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FURTHER STUDIES ON THE SUBTERRANEAN ALGAL FLORA OF THE MISSOURI BOTANICAL GARDEN

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Historical Introduction

Although it has long been recognized that the surface of soil forms a very suitable habitat for many algae, especially the *Cyanophyceae*, it is only in comparatively recent times that investigators have realized that these small plants may possibly play an important part in the biology of the soil. The history of the investigation of soil algae is therefore of comparatively recent date, and, as contrasted with that of the other soil organisms, such as bacteria or protozoa, our knowledge of the algal flora of soil is very imperfect. It seems possible that the presence of these autotrophic plants in the soil may be of great importance, and that their physiological processes may greatly influence soil conditions and also have important effects on the soil as a medium of growth for the other soil organisms. However, any such influence exerted by soil algae is yet to be proved, for we are still in complete ignorance on this matter.

The literature bearing on the problem of soil algae, either directly or indirectly, is nevertheless very extensive although confined to two or three main lines of investigation, namely, the

systematic study of soil algae, their relation to nitrogen, whether or not they are capable of fixing atmospheric nitrogen, and lastly, an immense amount of work has been done on the physiology of algae, their relation to light and organic food substances, which is very important in the soil algae question. Since no very complete review of this literature exists, it is desirable that a summary of the more important papers be made here. The three phases of the literature mentioned above will be taken up separately.

THE SYSTEMATIC STUDY OF SOIL ALGAE

Although the systematic examination of the algae occurring in or on the surface of the soil was not undertaken for many years after the development of the soil algae problem, it will possibly be best to begin with this phase in order that some idea may be obtained of the nature of the organisms concerned.

The first extensive work relating to the soil algal flora was published by Esmarch ('10) and dealt with samples taken chiefly from soil in German African colonies. Esmarch's method of culturing his soil samples was such as to favor the growth of *Cyanophyceae*, and he confined himself almost exclusively to the consideration of these forms. The work first published by him was not undertaken primarily as an investigation of soil algae, but rather to increase the knowledge of the distribution of the *Cyanophyceae*. The soil samples fell into two main groups as regards the depth from which they were taken, namely, 1–25 cm. and 25–50 cm. Altogether, between 30 and 40 species were identified, chiefly species of the *Oscillatoriaceae* and *Nostocaceae*. In considering his data Esmarch came to the conclusion that the majority of the species occurred in the samples from the upper 25 cm., and that a greater proportion of the samples from the lower depths produced no growth. He was stimulated by these results to take up in greater detail the study of soil algae, with the result that in 1914 he published a second paper dealing with extensive investigations of the soil algae in Germany. Esmarch's second paper deals chiefly with the relation of the soil algal flora to depth and to cultivation. With regard to cultivation, surface samples taken from cultivated soils almost always produced a

good growth of algae, whereas very few samples from uncultivated soils produced algae except damp sandy soil from the shores of the river or lakes. At 10–50 cm. below the surface no algae could be obtained from soil samples from uncultivated soils, while samples from a similar depth in cultivated soils frequently produced a growth, though fewer species were represented than at the surface. Esmarch felt that since in cultivated soils the lower strata contained approximately the same species as the surface, with the exception that fewer species were present, the relation between the two levels might be such that the lower layers derive their flora directly from the surface in the operations of cultivation, such as ploughing or by the action of earthworms or of seepage water. Esmarch experimented also on the effect of prolonged darkness on some of the algae isolated by him from the soil, but was unable to produce any conclusive proof that they are able to persist for any great length of time in total darkness.

Robbins ('12), investigating the algal flora of the surface and first few inches of soils in Colorado with special reference to their extraordinary nitrogen-fixing capacity, in spite of their low content of organic matter, recognized about 21 species of algae, chiefly *Cyanophyceae*, but including two *Chlorophyceae* and a diatom. He believed that the abundant blue-green algae formed a source of carbohydrate food for the nitrogen-fixing organism, *Azotobacter*, and that for this reason the bacteria were able to flourish in quantity in spite of the low organic content of the soil.

Petersen's work in 1915 deals chiefly with diatoms growing on the surface of the soil, but some attention is also given to *Chlorophyceae*, although the *Cyanophyceae* are entirely ignored.

Bristol ('19a) gives an interesting account of algae obtained from soil samples preserved in an air-dry condition for many years and extended our knowledge not only of the degree of resistance to desiccation of these forms, but also the range of species of the soil algal flora. Two years later the same author (Bristol, '21) published a more extensive soil flora. From an investigation of 50 soil samples taken from the surface 6 inches of soil she identified 20 species of *Chlorophyceae*, 24 *Cyanophyceae*, and 20 diatoms. This must be regarded as the most complete algal flora of the soil published so far. The *Chlorophyceae* included chiefly unicellular

forms, although *Bumilleria exilis* and *Ulothrix subtilis* were almost constantly present; the *Cyanophyceae* were largely species of *Nostoc*, *Anabaena*, and *Phormidium*, and the diatoms, *Navicula* and *Nitzschia* spp.

Moore and Karrer ('19), taking up again the investigation of the lower strata of the soil as initiated by Esmarch, established the existence of diatoms and other algae at depths up to 100 cm. below the surface of the ground, the greatest depth at which such organisms had been recorded. The interesting fact was discovered that a unicellular green alga which they identified as *Protoderma viride*[1] is constantly present as far down as 1 m. below the surface. Repeating Esmarch's subterranean culture method, in a slightly varied form, they found that this algae could live and remain green when sunk into the ground for a period of 4–5 months.

The Relation to Atmospheric Nitrogen

By far the greatest amount of work done so far has been concentrated on the relation of the algae to nitrogen and especially to atmospheric nitrogen.

Even in the early fifties following the work of Boussingault and his contemporaries on the nitrogen relations of the higher plants, Laurent ('54) and Morren ('54) came independently to the conclusion that lower organisms, including protozoa and algae, are unable to make use of atmospheric nitrogen, but pass into a resting condition when the supply of combined nitrogen becomes depleted in the medium in which they are living.

For more than 30 years no further work seems to have been done until the opposite opinion was put forward by Frank ('88), who discovered that if soil is kept moist with distilled water there is an increase in the nitrogen content after standing for several months. Frank's attention was drawn to the fact that the increase in nitrogen was not present in the form of nitrates, but in an organic form. Furthermore, on the surface of the soil he noticed that a mat of algae had developed, including *Oscillatoria* spp., *Chlorococcum humicola*, and others. Frank thus came to the conclusion that atmospheric nitrogen is transformed into nitrates

[1] This now appears to be identical with *Chlorococcum humicola* (Näg.) Rab. as recorded by Bristol ('19b, '21) and others.

by the algae in the soil, and that these are later elaborated into a complex organic form.

In the same year Gautier and Drouin ('88), working with soils containing nitrogen only in an ammoniacal form, found that such soils, if poor in organic matter, show a lower nitrogen content after standing, but that a higher nitrogen content will result if much organic matter is present. In all cases, however, irrespective of the organic content of the soil, the ammoniacal nitrogen decreases and the organic nitrogen increases, the latter in direct proportion to the strength of the algal mat which develops on standing. These workers therefore drew the somewhat original conclusion that the algae are important as conservers of ammoniacal nitrogen, whose escape from the soil they prevent, rather than as fixers of atmospheric nitrogen. They thought that the escaping ammoniacal nitrogen is absorbed by the algae and transformed into a more stable organic form.

In the following year Frank ('89) put forward what he considered to be direct proof of the nitrogen-fixing power of the lower algae by demonstrating that whereas a substantial increase in nitrogen content takes place if soil is kept moist and exposed to daylight, no increase takes place in the same soil if it is kept in the dark, or sterilized. Either of these last two treatments prevents the growth of algae, while the darkness, although excluding the algae, permits growth of bacteria. Frank believed, therefore, that the increase of nitrogen taking place in the light was due entirely to the growth of algae.

The work of Schloesing and Laurent ('92) lent further support to Frank's idea. These workers used poor subsoil and sand for their substratum, and inoculated with impure suspensions of algae, adding also a small sample of ordinary soil extract. They kept their soil in a confined atmosphere and had an arrangement for determining the actual change in the gaseous nitrogen contained in that atmosphere. In this way they were able to check up the loss of gaseous nitrogen with the amount of nitrogen increase in the soil. With *Nostoc punctiforme* and *Cylindrospermum majus* they found a substantial increase in the nitrogen content of the soil, and they also established the fact that there is a greater increase of nitrogen in the upper layer of the soil con-

taining the algae than lower down. While realizing that bacteria possibly play a part in the increase of nitrogen, they believed that the algae, being present in larger numbers, are responsible in a greater measure for the fixation of atmospheric nitrogen which occurs.

In 1893, Koch and Kossowitsch kept sand cultures inoculated with a suspension of algae in daylight, and similar cultures as controls in darkness. Their experiments served to confirm the conclusions of Frank that in the presence of light active fixation of atmospheric nitrogen takes place, but that if the growth of algae is prevented by absence of light no fixation occurs.

Since this time there have been repeated investigations of the activities of algae in association with soil bacteria with special reference to nitrogen relations. One of the most energetic workers in this line was Bouilhac. In 1896, claiming to have isolated in pure culture certain algae, this worker found that *Nostoc punctiforme*, *Schizothrix lardacea*, and *Ulothrix flaccida*, when free from bacteria, were quite unable to live in a solution containing no nitrogen. *Nostoc punctiforme* could live perfectly well under these conditions provided that a drop of soil extract containing soil bacteria was added, and, moreover, the solution would show a decided increase in nitrogen content, showing that fixation of atmospheric nitrogen had taken place. The other two species, however, were incapable of living under similar conditions. Later, in 1897 and 1898, he showed that *Nostoc punctiforme* could live in association with soil bacteria in the absence of combined nitrogen, and also in darkness, provided that glucose is supplied. Without glucose no growth takes place in darkness. A very interesting experiment was recorded by the same worker in 1903, in which he showed that algae and soil bacteria, inoculated together into sterile sand with mineral nutrients devoid of combined nitrogen, are capable of fixing enough atmospheric nitrogen to support the growth of higher plants.

The idea of a symbiotic relationship between algae and soil bacteria has since gradually grown in popularity. Reinke ('03b) believes that *Volvox* colonies are ordinarily infested with the nitrogen-fixing organism *Azotobacter*, that the bacteria obtain some carbohydrate food from the gelatinous matrix of the colony,

and in return give up some combined nitrogen, in which they are very rich. In a somewhat earlier paper the same author (Reinke, '03a) extends this idea to marine algae, and expresses the probability that here also the algae are dependent upon *Azotobacter* for their nitrogen supply, and that the bacteria live embedded in the gelatinous surface of the algae.

Fischer ('04) asserts that there is a similar symbiosis between a terrestrial species of *Oscillatoria* and *Azotobacter*, and that even when the bacteria are not readily visible they can be made to increase rapidly by culture in 1 per cent mannite solution. It is interesting to note that he was unable to obtain the bacteria from *Hormidium parietinum* and *Pleurococcus vulgaris* inhabiting the bark of trees.

In opposition to all the work enumerated above and many other similar investigations too numerous to mention, all dealing with impure cultures or with algae and bacteria in mixed culture, indicating an increase in nitrogen content of the culture medium which was sometimes erroneously attributed to the algae themselves, is a group of articles dealing with experiments on algae in pure culture, free from bacteria, which give more conclusive evidence of the relation of algae to atmospheric nitrogen.

The earliest of these workers with pure cultures was Kossowitsch ('94) who isolated an alga from soil and proved quantitatively that no fixation of nitrogen could be demonstrated in the case of the pure alga, but that if impure cultures containing bacteria are used considerable fixation takes place. Kossowitsch realized the possibility of a symbiotic relationship.

Krüger and Schneidewind ('00) carried out very extensive experiments from which they drew similar conclusions. Working with about 8 species of *Stichococcus*, as many of *Chlorella*, and about 6 species of *Chlorothecium*, they proved that not one of these is able to live in a solution containing no combined nitrogen, and that even when growing vigorously there is no fixation of atmospheric nitrogen. They attribute the increased fixation in mixed cultures to the beneficial action of the symbiotic relation between the algae and bacteria rather than to the activity of the algae themselves.

Charpentier ('03), working with *Cystococcus humicola*,[1] also came to the conclusion that in pure culture this species is incapable of fixing atmospheric nitrogen.

Heinze ('06), working with a species of *Nostoc*, claims to have proved that fixation of nitrogen occurred in this species, but his alga was slightly infected with a species of *Streptothrix*. After isolating the fungus Heinze proved that this, by itself, was unable to fix atmospheric nitrogen, whereas the alga with its slight infestation showed a decided fixation. Heinze thus considered it proven that the fungus played no part in the nitrogen fixation, which he thought was due entirely to the alga. Since this worker confesses that he was unable to free his alga from the fungus, it is possible that bacteria were also present in the culture.

Schramm ('14), who gives a very complete survey of all the earlier work on the subject, working with 7 diverse species of soil algae in pure culture, proved that when no combined nitrogen was provided, not one of these algae was able to grow. This seems to indicate that they are incapable of making use of atmospheric nitrogen.

Nakano ('17), in the course of his paper, gives a more thorough investigation of the symbiosis between *Azotobacter* and algae than any other worker. He used species of *Chlorella*, *Scenedesmus*, etc., in pure culture, and also isolated and named from soil a pure strain of *Azotobacter*. Using a solution containing no combined nitrogen and .5 per cent glucose, Nakano found that the algae were unable to produce any growth, and moreover fixed practically no nitrogen when grown in pure culture in such a solution. *Azotobacter*, however, grew well under the same conditions and fixed atmospheric nitrogen abundantly. Mixing the pure *Azotobacter* and the pure algae in the same culture solution, a good growth of algae resulted, and in addition the amount of nitrogen fixed by the mixed culture of *Azotobacter* and algae was 20 per cent higher than by the pure strain of *Azotobacter* alone. This clearly proves the beneficial effect of the association of the two organisms. In further discussion concerning the more exact nature of the symbiosis, Nakano comes to the conclusion that the algae live at the expense of nitrogenous compounds derived from

[1] Probably identical with *Chlorococcum humicola* (Näg.) Rabenh.

the *Azotobacter*, which they obtain after the death and autolysis of the bacterial cells. A sterilized culture of *Azotobacter* is not capable of furnishing the nitrogen necessary for algal growth, since the nitrogen is unavailable in a complex organic food, and enzyme action is destroyed. Debating on the conditions in nature, Nakano is inclined to believe that the symbiosis is not of any real importance to the algae, for he argues that whereas many thousands of *Azotobacter* cells are necessary to supply nitrogen for a single algal cell, one never sees algae thus infested in nature, and he is very doubtful whether *Azotobacter* could supply the nitrogen required by the larger seaweeds. In explaining the beneficial action of the algae on the nitrogen-fixing capacity of *Azotobacter*, Nakano thinks that possibly the oxygen evolved by the algae during photosynthesis is responsible for this. *Azotobacter* is more active if grown in thin layers than in thick layers and thus it seems to have a high oxygen requirement. The presence of algae might therefore be helpful so long as photosynthesis is going on.

In the past few years very few workers have claimed that algae by themselves are capable of fixing atmospheric nitrogen. Benjamin Moore and Webster ('20), and later Benjamin Moore, Whitley, and Webster ('21) have performed certain experiments and claim that nitrogen fixation is possible in both freshwater and marine algae. They made no attempt, however, to free their cultures from bacteria, and moreover they were apparently working in complete ignorance of the extensive literature on the subject and the results of the many workers who had preceded them. They were apparently also unaware of the fact, long proven at that time, that a symbiotic relationship exists between nitrogen-fixing bacteria and algae. Their results cannot therefore be considered to add anything to our knowledge of the relation of green algae to atmospheric nitrogen, although their philosophic discussions are interesting.

Wann ('21), on the other hand, used only pure cultures, and he also claims that nitrogen fixation is possible under certain conditions in green algae. Using 7 different species, he supplied his algae with nitrogen in several forms, in organic combination or as ammonium or calcium salts, in series with and without glucose.

Analysis was made of the media before the experiment, and of the culture solutions at the conclusion, by the Gunning-Kjeldahl method, and it was found that where the nitrogen was provided in the form of nitrate and in the presence of glucose, there was an increase in nitrogen content amounting to as much as 54 per cent. The author therefore concludes that the algae had fixed atmospheric nitrogen to that amount. Wann's work occupies a very isolated position, being the only case in which fixation of atmospheric nitrogen by green algae in pure culture has been claimed.

Muenscher ('23), working on the nitrogen metabolism in *Chlorella*, states incidentally that his results do not conform with those of Wann and that he always recovered the same amount of nitrogen after the experiment as was provided at the beginning. He was unable to demonstrate nitrogen fixation in this species.

Wann's work has been criticized by Bristol and Page ('23). These workers have carefully repeated Wann's experiments, and find that there is no fixation of atmospheric nitrogen by the algae used by them. They claim to have found the weak point in Wann's work to be the method of analysis when nitrates are present. They seem to prove their point fairly conclusively, and as far as one can judge from the evidence at present it seems quite likely that Wann's results will have to be held in abeyance until an explanation can be given of why his results are at variance with the work of other investigators.

Summarizing our knowledge of the relation of algae to nitrogen, it seems to be fairly well established that green algae in pure culture, free from bacteria, are unable to fix atmospheric nitrogen, but that when cultivated in association with *Azotobacter*, their presence seems to be beneficial, so that the capacity of the bacteria for fixing nitrogen is stimulated. The relation between the two organisms is probably of a symbiotic nature, the bacteria deriving carbohydrate food material from the gelatinous sheaths of the algae, and the algae thriving on the nitrogenous material provided by the bacteria.

The Relation to Light and Carbon

In the consideration of the peculiar conditions in which algae must live if they are present beneath the surface of the soil in an

active condition, their relation to darkness and capacity for saprophytic nutrition must be important. According to Bristol, the number of algae in the soil at 4 inches below the surface is nearly as great as in the surface inch. Furthermore, the quantity is far greater than usually supposed. "Taking 100,000 as a rough estimate of the number of algae per gram of manured soil in a given sample and assuming the cells to be spherical and of average diameter, 10 μ, it has been calculated that the volume of algal protoplasm present was at least three times that of the bacteria, though only one-third that of the protozoa." [Russell, The Micro-Organic Population of the Soil, p. 110. 1923.]

Such great numbers of algae living at the depths at which they are found must be in complete darkness and under conditions which render photosynthesis impossible. A considerable amount of work has been done on the physiology of the algae, and the question of their capacity for saprophytic nutrition in the absence of light has received ample attention. There is so much literature on the subject that only a few of the cases in which saprophytic nutrition has been demonstrated can be mentioned.

Radais ('00), working on *Chlorella vulgaris*, found that his cultures remained green in darkness and also that when grown on potato malt agar, growth was equally good in light and in darkness, while Grintzesco ('03), working with the same organism, obtained exactly the same results, and even states that with 2 per cent glucose, growth in total darkness may be much better than in the light. Artari ('06) and Kufferath ('13) also support these statements.

Charpentier ('02, '03) found that *Cystococcus humicola*[1] grew and remained green in darkness, but that the yield was much greater in the light, a weight of 330 mgms. resulting in the light as against 27 mgms. in darkness. Artari ('02) also found that the same alga produced normal healthy growths if grown in darkness with 1 per cent mannite, lactose, glucose, levulose, or canesugar.

Treboux ('05) has shown that other substances than carbohydrates may be used by algae as a source of carbon in the absence of light, for he obtained growth with several species of algae in

[1] Probably *Chlorococcum humicola* (Näg.) Rabenh.

darkness, using the potassium salts of various organic acids and also the amino derivatives, such as glycocoll, alanin, leucin, and asparagin. With the acids, the simplest compounds seemed to be the most easily assimilated, and a concentration of .25 per cent acetic acid was used by practically all the 40 species of algae investigated.

Chlamydomonas Ehrenbergii, according to Artari ('14), is able to use glucose in darkness, for if glucose is present the yield in the absence of light is 20–25 times greater than if no sugar is given. However, the growth is at no time so good as when autotrophic nutrition is permitted.

Dangeard ('21) records that he has grown *Scenedesmus actatus* in darkness for 8 years. One per cent glucose and .8 per cent peptone were provided, and frequent transfers to new media were made. After being subjected to darkness and prevented from exercising its photosynthetic function for so long, exposure to light resulted in the evolution of oxygen in 5 hours. This is very interesting in connection with the soil algae question.

The above work, confined to species of the *Chlorophyceae*, is altogether in favor of the possibility of heterotrophic nutrition in darkness. Not so much work of this kind has been done on the *Cyanophyceae*, and the evidence is not nearly as conclusive. Bouilhac ('97) found that *Nostoc punctiforme* would grow in darkness provided that glucose was present. His experiment, however, is complicated by the fact that he was using a mixed culture with soil bacteria. Pringsheim ('13) isolated several species of *Nostoc* and *Oscillatoria*, but found that the addition of organic matter to the cultures produced deleterious effects if used in large quantities, and that the stimulating action of small amounts was never very striking. He was unable to demonstrate that saprophytic nutrition with organic food was possible in the dark.

Cultural Methods and Results

The culture vessels were prepared in essentially the same way as described in an earlier paper (Moore and Karrer, '19) except that the sand was well washed before being used and the culture solution was full strength. It was not felt necessary to slant the

bottles, since the sides of the bottle served as a suitable substratum for forms not requiring aquatic conditions. All the samples were taken from excavations in the Missouri Botanical Garden in localities where the soil had not been disturbed for at least twenty-five years, so that the results obtained should be regarded as pertaining to uncultivated rather than to cultivated soils. For the most part the several localities had been covered with a dense turf. The depth from which the samples were taken was noted at the time, and every precaution observed to prevent contamination of the samples with soil from a different level. In the case of the first 3 series of cultures the culture vessels were inoculated in the garden with an unknown amount of soil, but in the fourth series the samples were taken in sterile bottles, and inoculation was performed in the laboratory with weighed amounts of soil. In order to prove that infection from the air did not take place during the weighing of the inoculum, a sterile control culture was left exposed to the air of the laboratory for 36 hours during the weighing of the soil, but no growth of algae resulted from this exposure. The algae developing in the cultures can therefore be assumed to have developed from the soil with which they were inoculated.

The first 3 series of cultures were inoculated with soil of different levels down to 4 or 5 feet, but in the last series the samples were taken as deep as 9–10 feet.

The results recorded here can be considered as amplifying the data given in the earlier paper (Moore and Karrer, '19), not only in extending our knowledge of the depth at which algae occur in the soil but also in giving an idea of the variety of the subterranean flora. Although by no means as luxuriant as at the surface (as investigated by Bristol, '19a, '21), the flora nevertheless includes a far greater number of species, especially *Chlorophyceae*, than one would expect in subterranean conditions.

The species which is almost universally present is *Chlorococcum humicola*, recorded as *Protoderma viride* in the earlier paper. It should be noted that Bristol ('19b, '21) has obtained this alga from the Malay States, and has also found it usually present in English soils. It therefore seems likely that this species is universally present in all soils. In the present cultures it appeared in almost

every case where a rough inoculum (about 10 gms.) of soil down to 3 feet was used, and it will probably occur in samples as small as .1–.2 gms.[1] Below 3 feet, however, its occurrence is so uncertain that one cannot be sure of obtaining it unless more than 5 gms. of inoculum are used.

Chlorococcum humicola is accompanied by a number of other green algae, which seem to inhabit the soil in somewhat smaller numbers than this dominant species, so that some, but not all, may occur along with *Chlorococcum* in most of the cultures. Below 3 feet it not infrequently happens that one or other of these species may be obtained in a unialgal culture, a circumstance which aided considerably in their identification as distinct species.

Whereas *Chlorococcum humicola* occurs with sufficient constancy for it to be possible for us to come to some conclusions regarding its numerical distribution in the lower layers of the soil, most of the other species seem to be very uneven in their distribution and to occur without any regularity, so that it is impossible to make any definite statements concerning their numbers in the soil.

It is noteworthy that these accessory species do not correspond very closely to the list of *Chlorophyceae* enumerated by Bristol ('21) as accompanying *Chlorococcum humicola* in the English soils. One species which is given by her, namely, *Chlorochytrium paradoxum*, is, however, more constantly present in the Missouri samples than in England. Apart from this, there is almost no conformity between the two lists, and one is forced to the conclusion that the subterranean flora is probably as variable as the surface flora.

The most interesting species isolated from the Missouri soils and not present in Miss Bristol's list is *Protosiphon botryoides*, nearly always present in our cultures (and possibly "*Cladophora* sp." in Moore and Karrer, '19). This species is not native in England and is therefore not likely to occur in the subaerial flora. The frequent occurrence of *Chlorella* in our cultures, and its absence from the British list is, on the other hand, somewhat surprising, and one cannot help thinking that possibly Miss Bristol

[1] The weight of inoculum must only be regarded as approximate, since the moisture, which varied in samples from different levels, was not taken into account.

overlooked it, or mistook it for small cells of *Chlorococcum humicola*.

Botrydiopsis arhiza is another species which is probably more often present than is apparent from the accompanying lists, since isolated cells are easily overlooked. As regards the species of *Chlamydomonas*, it is possible that Miss Bristol has included at least one species in her stages in the life history of *Chlorococcum humicola* ('19b). The form figured by her in pl. 18, figs. 27, 28, is very similar to the alga recorded here as Species B. *Chlorococcum humicola* has been isolated by one of us in pure culture, and a palmelloid stage has never been observed to occur. Species B has also been isolated, and it always retains its characters both on solid and liquid media.

Species of *Cyanophyceae* and diatoms are notably much fewer than recorded in Miss Bristol's surface flora, and the *Cyanophyceae* only occurred in dominating numbers as a rule in the upper 18 inches of soil. On the other hand, the development of *Lyngbya subtilis*, 2½ feet down, in Series A, is suggestive of the possibility that in the method of culture used the development of *Chlorophyceae* was perhaps unduly favored. The obtaining of a pure culture of *Oscillatoria amphibia* from a depth of 8 feet 2 inches in Series D is likewise a very interesting fact, which was considered at first as being due to a chance contamination from a different level. However, since the greatest precautions were taken during the collecting of the samples and the inoculation of the cultures, and, moreover, since isolated cultures of different species were obtained from samples of soil taken from 5 to 9 feet in about 8 different cultures, it seems unlikely that foreign infection was responsible for all 8 cultures. This is especially true since with one or two exceptions the constituents in these cultures are not at all common in the cultures from higher levels, and represent in some cases the only record of that particular species. If the development of these cultures from lower levels is due to infection with soil from a higher level, the species most commonly occurring there, namely, *Chlorococcum humicola*, would be expected to appear.

The diatoms are not fully recorded here for the first 18 inches, since a few other species sometimes occurred in the uppermost

samples taken, but in too small numbers for the cleaning up of a sample for specific determination to be a successful operation. The records for the lower depths can be considered fairly accurate. The record of *Navicula atemoides* in pure culture at a depth of 5 feet 5 inches is extremely interesting, and this is the species most constantly present in cultures from the lower strata.

In identifying the species the greatest difficulty was experienced in deciding between *Chlorochytrium paradoxum* and *Protosiphon botryoides*, and it was often impossible to determine whether one or both of the species were present. *Protosiphon*, after a length of time in culture, proceeds to form large resting aplanospores which may be released from the old mother cell-wall and remain for a long period as orange-colored cysts. *Chlorochytrium paradoxum* also forms similar large cysts which cannot be distinguished from those of *Protosiphon*. The records for these two species are therefore somewhat uncertain.

The succession of species in the cultures was very interesting, for with age, species originally dominating would fade away and give place to others. *Protosiphon* begins to lose its typical form after about 6 months' culture, probably because an aquatic habitat is not normal for it. The most interesting case, however, is that of the alga recorded as Species A, which was never observed until a culture reached the age of 8–12 months, except in the culture 42C where it occurred as a unialgal culture and formed a recognizable but feeble growth in 3 months. Probably high organic food requirement is responsible for these facts.

It should be noted that series D produced a large number of species which were not observed at all in the three earlier series. Possibly this is due to the fact that a greater proportion of unialgal cultures occurred in this series. These rarer species may have been present in the other series, but were not conspicuous because of the greater mass of other forms, or again the difference may be due to a local variation in the flora.

Notes on the Species Observed

CYANOPHYCEAE

Nostoc commune Vauch.

Forti in De Toni, Syll. Alg. 5: 404. 1907.

This species occurred in a well-developed form in several cultures, all above 1 foot 4 inches. The trichomes were about 5 μ in diameter, with heterocysts very slightly larger than, or equal in size to, the vegetative cells. Spores were abundant in long chains and measured about 6 × 8 μ.

Nostoc muscorum Ag.

Forti in De Toni, Syll. Alg. 5: 400. 1907.

This species was identified in only one culture, from a depth of 6 inches. The spores were somewhat immature, but the other characters seemed sufficient to identify it.

Nostoc comminutum Kütz.

Forti in De Toni, Syll. Alg. 5: 393. 1907.

A pure culture of an alga corresponding to this species was obtained from soil at a depth of 3 feet 6 inches. The colonies were small and distinct from each other, consisting of trichomes 3–3.5 μ in diameter, closely convoluted. Heterocysts 6.5 μ in diameter were observed, but spores were wanting. The occurrence of a blue-green alga at such a depth is somewhat surprising.

Phormidium tenue (Menegh.) Gom.

Forti in De Toni, Syll. Alg. 5: 227. 1907.

This species was identified in only one culture, at a depth of 1 foot. It was quite typical.

Phormidium molle (Kütz.) Gom.

Forti in De Toni, Syll. Alg. 5: 219. 1907.

In the same culture as the preceding species was a small quantity of a very moniliform *Phormidium* with trichomes 3.5 μ in diameter, which seemed to correspond fairly well with the description given for this species.

Lyngbya subtilis West

Forti in De Toni, Syll. Alg. 5: 285. 1907.

In a culture of soil from a depth of 2½ feet there developed after a time a quantity of a very slender blue-green alga with a distinct sheath. The filaments were about 1.5 μ in diameter, and the trichomes themselves barely 1 μ. The alga seemed nearest to *Lyngbya subtilis* West.

Oscillatoria amphibia Ag.

Forti in De Toni, Syll. Alg. 5: 169. 1907.

The development of a pure culture of this species from soil of more than 8 feet depth was a very surprising phenomenon. The alga was in every way normal and typical.

BACILLARIEAE

Navicula atemoides Grun.

Van Heurck, Diat. 227. *pl. 5, f. 230.* 1899.

This was the most general of all diatoms, and was a general constituent of the flora from the surface 6 inches to a depth of 5 feet 5 inches, which is the lowest record obtained for it.

Navicula mutica Kütz.

Van Heurck, Diat. 206. *pl. 4, f. 167.* 1899.

This species was not quite as frequent as the preceding one, but nevertheless has been found at a depth of 4 feet.

Hantzschia amphioxys (Ehr.) Grun.

Van Heurck, Diat. 381. *pl. 15, f. 483b.* 1899.

This is one of the less frequent diatoms.

Nitzschia palea (Kütz.) W. Sm.

Van Heurck, Diat. 401. *pl. 17, f. 554.* 1899.

Rather more frequent than the preceding species and observed below 4 feet.

CHLOROPHYCEAE

Chlorococcum humicola (Näg.) Rab.

Bristol in Jour. Linn. Soc. Bot. **44**: 473. 1919.

This alga developed in nearly every culture down to 3 feet, and the lowest record is 5 feet 5 inches. As will be seen from table IV, series D, .01 gm. of soil at 12 inches depth gave a good growth of *Chlorococcum*. It can therefore be assumed that at this depth there are at least 100 individuals of the alga to the gram. At 3 feet depth, however, they may be as sparse as one individual in 2 gms., and at 4 feet 2 inches rarer than one in 5 gms. The alga reproduced freely by both zoogonidia and aplanospores, and the vegetative cells readily formed orange resting cysts when conditions became unfavorable for vegetative growth. There is some doubt as to whether the large cells mentioned by Bristol ('19b) really belong to this species. They possibly belong to *Chlorochytrium*. Furthermore, the palmelloid stage mentioned and figured by Bristol probably belongs to a species of *Chlamydomonas*, Species B of this work (*vide supra*).

Chlorochytrium paradoxum (Klebs) West

Bristol in Jour. Linn. Soc. Bot. **45**: 8. 1920.

This species was a fairly frequent constituent, though it was often difficult to decide for certain reasons whether it was really present or not, especially when *Protosiphon botryoides* was also present (*vide supra*). It usually occurred in the form of large olive-green cells or bright orange resting cysts, reaching a diameter of 100 μ. There were rarely any of the smaller cells showing the characteristic cytological structure of the genus, and the only way in which the identity of the cysts was suspected was in following the development of the zoogonidia into the characteristic vegetative cells. Large aplanosporangia with a few comparatively large cysts of unequal size were sometimes observed, all in a resting condition. The thickness of the wall of the aplanosporangium might be as much as 7–10 μ.

Protosiphon botryoides (Kütz.) Klebs

Klebs, Bed. Fortpflanz. 169–222. *pl. 1, f. 1–16.* 1896.

This species was a very frequent constituent of the cultures, being present in practically all samples in series B and C. Very often it occurred in great abundance, so that it was probably present in considerable quantity in the soil. In the cultures the alga was not always typical in form because of the aquatic conditions but it was always easily recognized. Reproduction by aplanospores of varying size was frequent, and the production of gametes, their conjugation, and the formation of the tiny star-like zygotes figured by Klebs (*loc. cit., pl. 1, f. 16*) were also observed. Sometimes the aplanospores were transformed into large, orange, thick-walled resting cysts. The lowest depth from which the species has been obtained is 4 feet.

Chlorella spp.

The genus *Chlorella* was represented fairly constantly in the cultures, and it seems quite possible that more than one form occurs. The specimens isolated from a certain culture measured uniformly 3–5 μ in diameter, whereas in other cultures they were only 2–3 μ in diameter. This indicates that possibly two forms occur in the culture, but in the records no distinction is made between the two. *Chlorella* has been observed as far down as 5 feet.

Trochiscia reticularis (Reinsch) Hansg.

West, Brit. Freshw. Alg. *f. 82K.* 1904.

This species occurred in 3 of the 4 series of cultures, and in the last series, D, was almost as constant and as abundant a constituent as *Chlorococcum humicola* itself, the indications being that there are more than 100 specimens per gram at a depth of 12 inches. It must be noted, however, that whereas .01 gm. of soil from this level produced individuals of *Trochiscia*, 5 gms. from the same level failed to produce any specimens. The distribution of this species therefore seems to be very uneven. The specimens varied in size from 10 to 20 μ in diameter, and were sometimes oblong rather than spherical in outline, with the dimensions 12 × 8 μ. The lowest record for the species is 3 feet 9 inches.

Trochiscia sp.[1]

?Acanthococcus sp., Reinsch, Ber. d. deut. bot. Ges. 4: 243. *pl. 12, f. 18.* 1886.

This species was observed only in a single culture, at a depth of 3 feet 9 inches. It was sharply distinguished from *Trochiscia reticularis*, which was the more common species of the genus, by its short, blunt, and rounded projections. The specimens varied in size from 14 to 18 μ in diameter and seemed nearest to the form recorded by Reinsch as *Acanthococcus* sp.

Dactylococcus sp.

Cells of this form were observed only in two cultures. They were stout and spindle-shaped with slightly acute apices, and measured about 12 × 8 μ. A pyrenoid was distinctly present, and reproduction was observed by the formation of 4 similar individuals within a mother cell, which might at this time reach the size of 12 × 16 μ. In general appearance the form was most suggestive of the *Dactylococcus* state of *Scenedesmus* figured by Grintzesco ('03, p. 217), but what it really is was not decided. Bristol ('21, p. 74) records a species of *Dactylococcus* from English soils, but there can be no confusion between the form observed here and her species.

Ulothrix subtilis Kütz.

West, Brit. Freshw. Alg. 76. *f. 20 C-F.* 1904.

This species is not at all a frequent constituent of the subterranean flora, although, according to Bristol ('21), it is almost universally present on the surface. In two of the series of cultures it did not occur at all, and its occurrence in the other two series was sporadic. The record at 3 feet in series D is somewhat surprising. It seems probable that for some reason this alga does not usually descend very far from the surface of the soil, possibly owing to the fact that its zoogonidia are not long motile. The filaments were 5–7.5 μ in diameter, and the cells almost as long as, or a little longer than, broad. There was some tendency for the filaments to break up into short lengths.

[1] Bristol ('21) records two species of *Trochiscia* from English soils, but neither seems to be identical with the forms observed here.

Stichococcus bacillaris Näg.

West, Brit. Freshw. Alg. 80. *f. 24A*. 1904.

This species does not seem to occur with any regularity, unless, as is quite possible, the tiny cells were often overlooked. It was observed in only 3 cultures, the lowest record being 2 feet 6 inches.

Stichococcus scopulinus Hazen

Hazen in Mem. Torr. Bot. Club **11**: 161. *pl. 22, f. 4–6*. 1902.

This alga was observed in only two of the series and never below a depth of 12 inches. The cells were 3–4 μ in diameter and 11–16 μ in length. The filaments were often of considerable length and showed little tendency to dissociate. It seems likely that the occurrence of this species in the subterranean flora is dependent upon its local distribution at the surface and that it probably never descends to any great depth.

Uronema confervicola Hazen

Collins, Green Alg. N. Am. 88. *f. 66*. 1909.

The occurrence of a few isolated filaments of this species in 2 cultures from a depth of 3 feet was indeed surprising. The filaments were about 4 μ in diameter and of great length. The cells were provided with 2 pyrenoids each, and the apical cell was typically acute. The species is probably not a regular inhabitant of the soil but only a chance form. In both cultures it was present in such small quantity that a month after it was first observed it had quite disappeared.

Monocilia viridis Gerneck

Gerneck, Beih. Bot. Centralbl. II. **21**: 263. *pl. 12, f. 77–84*. 1907.

A form closely resembling this alga described by Gerneck in structure and appearance was observed in 3 cultures of series A. In the culture from the lower level, 3½ feet, it was present in considerable quantity and for a time favored the dominant constituent of the culture. Later it disappeared entirely from both cultures and left no trace. In the culture in which it had been most abundant, a considerable quantity of a yellow-green alga

appeared which was identified as *Botrydiopsis arhiza* Borzi. In this connection it is interesting to note that Gerneck states that *Monocilia viridis*, after being cultured for about 2 months in a liquid medium, loses its branched and filamentous form and goes into a unicellular palmelloid state. According to Gerneck, the filamentous form can only be obtained from the palmelloid stage by cultivating on a solid medium such as agar. The alga identified as *Botrydiopsis arhiza* in these cultures has been grown on agar, however, and it always retained its unicellular form. If Gerneck's observations are correct, therefore, it would seem that *Monocilia viridis* and *Botrydiopsis arhiza* as observed in these cultures are distinct from each other. It may be that *Monocilia viridis* is a more constant inhabitant of the soil than would appear from these records, but that it is not often in a recognizable condition.

Botrydiopsis arhiza Borzi

Borzi, Studi Alg. **2**: 169. *pl. 12, 13.* 1895.

This is the most regular representative of the *Heterokontae* in the subterranean flora. It is rarely present, however, in great abundance, and is possibly often overlooked. When there is not too much competition with other species it multiplies rapidly, however, and may form an abundant growth. Reproduction by aplanospores was very common, and zoogonidia with only one visible cilium were also observed. On one occasion biciliate swarmspores, similar to the gametes figured by Borzi, were seen, but no conjugation took place. The lowest depth from which the alga was obtained was 4 feet.

Characiopsis minuta Borzi

Borzi, Studi Alg. **2**: 152. *pl. 14, f. 1–12.* 1895.

The occurrence of this species in a single culture was somewhat surprising. It was present in considerable quantity in a sample taken from a depth of 2 feet 3 inches and only differed from the typical form in its slightly smaller size. The finding of this species seems to indicate that the spores of many algae may occasionally find their way into the soil and suggests that the subterranean flora may prove, with increased investigation, to be

almost as rich in species as the surface flora, though not in numbers of individuals.

Species A

The form recorded under this heading is a very problematical one which, since swarmspores have not yet been observed, cannot be properly identified. It is probably a fairly constant inhabitant of soil, but evidently requires very special conditions for its development, for it only appears in old cultures. The cells float freely in the water, quite isolated from each other and without any tendency to adhere in colonies. They are usually oval-oblong, 10–28 μ long by 7–18 μ broad, though quite frequently they may be spherical with a diameter of 9–15 μ. In some instances a number of unusually large individuals may occur scattered among the smaller ones, either spherical or oval, and reaching a diameter of 40 μ.

The most striking feature of the alga is the presence of a bright red spot in the interior of the cell. This is obviously not a stigma, for it is much larger, reaching a diameter of 2–8 μ. The chloroplast is a small parietal plate which only covers part of the wall. There is neither a pyrenoid nor starch present, though oil is abundant. The systematic position of the alga is unknown.

Species B

This is most probably a species of *Chlamydomonas*, and was the most frequently encountered representative of the genus. It is in all probability more constantly present than is indicated by the tables, and, especially in the earliest examination of series A, was possibly very often mistaken for stages of *Chlorococcum humicola*. Complete isolation of the form has shown, however, that it is a distinct species. It usually occurred as oval cells embedded in a gelatinous stratum, and if present in any great quantity, or if pure, forms a soft gelatinous stratum similar to masses of *Tetraspora*. A slight change in temperature causes the green cells to acquire cilia and to swim out of the gelatinous matrix. The period of swarming is comparatively short, and the cells soon become quiescent again and secrete quantities of mucilage to form a large expanded colony as before. Multiplication takes place in the palmelloid stage by the division of individual cells,

usually into fours. The motile cells are oval in form, 7–12 μ long by 3–7.5 μ wide. They have a massive bell-shaped chromatophore which only leaves a minute rounded clear space at the apical end of the organism. There is a distinct pyrenoid, usually in the posterior region, but sometimes more or less lateral. The stigma is distinct and somewhat elongated, lying in the apical region of the cell. The two cilia are about equal to the body of the organism in length. Bristol ('21) does not record the occurrence of this form, but figs. 27, 28 of pl. 18, in her work on *Chlorococcum humicola* ('19b), are identical in appearance with the forms observed in the present cultures, and possibly represent the same organism. It is noteworthy that this species has the record for depth, having been obtained at a depth of more than 9 feet.

Species C

This form is apparently another species of *Chlamydomonas*. It was only observed with certainty in a single culture in which it occurred pure. It may perhaps have been present in other instances, being possibly overlooked in the confusing mixture of other forms. The macroscopic appearance of the culture is very different from that of the preceding species, for the alga, instead of forming a large gelatinous stratum, as in Species B, produces small tough green flakes. Microscopically, the cells are somewhat rounded, possess a distinct pyrenoid, and are arranged in gelatinous clumps of greater or smaller size, though never forming such a large expansion, or possessing so much mucilaginous material as Species B. A slight change in the external conditions induces the development of cilia, as before, and the cells become motile and swim out of the gelatinous stratum. The motile cell is a little stouter than in Species B, reaching a length of 5–9 μ and a breadth of 3.5–6 μ. The chloroplast is not so distinctly bell-shaped; it leaves a larger and more irregularly shaped space clear at the anterior end, and the stigma is very minute and difficult to find, and also, when visible, is more anterior in position. The chief differences between the two species are to be found in the chloroplast and stigma and in the macroscopic nature of the colonies. It is possible that the *Protococcus*-like stages referred to above may belong to this species.

Species D

In many of the cultures gelatinous masses were frequently observed among the other forms, in which the cells embedded in the gelatinous stratum possessed the stigma and other characters of motile cells. The slightly changed conditions resulting from the removal of the sample from the large culture vessel, and its examination on a slide almost invariably induced the small cells to become motile. Then, in the earlier examination of the cultures, these motile cells were always observed to unite in pairs, producing a rounded zygote. An attempt was made, but without success, to follow the development of the zygote, and unfortunately the alga never occurred alone, or in sufficient quantity to make its isolation possible. In older cultures a similar form was frequently observed which agreed in size and in its conspicuous stigma, but the contents were so obscured by the presence of large starch grains that other cytological comparisons were impossible. Although in these older cultures the cells readily became motile as before, conjugation was not observed to occur.

The motile cells of this alga are smaller and more rounded than in the two preceding forms. They are $3\frac{1}{2}$–5 μ in diameter, and only slightly longer than broad. The chloroplast is not particularly massive, covering only a part of the external wall, and contains a pyrenoid which is usually quite conspicuous. The stigma is distinctly visible and the cilia are somewhat longer than the body length. The form has been observed as far down as 4 feet.

Species E

This is an additional species of *Chlamydomonas* which was not nearly such a constant constituent of the soil as some of the others, or at least it never occurred in quantities large enough to be conspicuous, although it may sometimes have been present as isolated individuals. It was never observed in a motile condition, but that it is normally a motile organism and probably a species of *Chlamydomonas* seem to be undoubted facts. It was readily distinguished from all other similar forms by its size, reaching a length of 12–21 μ and a breadth of 10–12 μ. The cells were usually broadly oval and occurred in most cases in the palmelloid form, each cell surrounded by its own distinct gelatinous envelope

which might reach a thickness of 10 μ, and aggregated in larger or smaller clumps. Very often active cell division seemed to have been taking place, for 2–8 smaller individuals were sometimes seen crowded together in the same envelope. In the majority of the larger individuals it could be clearly recognized that the alga was normally a ciliated organism by the differentiation of the cell contents, a clear apical region being easily distinguished. Apart from this, too much reserve food, both starch and oil, was usually present for the cytological structure to be clearly seen. The lowest depth at which it was found is 4 feet.

Species F

There was some doubt at first whether this organism is really an alga or a large bacterium, but the balance of evidence seems to be in favor of its being an alga, most probably of the genus *Stichococcus*. The cells are very minute, oblong and angular, 1½–2 μ broad and 4–5 μ long. They are distinctly, though faintly, green in color and seemed at first to have homogeneous contents. The higher magnification of the oil immersion showed, however, that in some individuals a clear space could be recognized either at one or both ends or else along the lateral margin. This seems to indicate that there is a chloroplast in the form of an extensive parietal plate. There is no pyrenoid, and very little blackening with the addition of iodine. The bacillus-like form of the organism at once distinguishes it from *Stichococcus bacillaris*. Information concerning its reproduction is to be desired before its exact affinities can be decided. The species was observed only in one or two isolated cultures.

Species G

This is one of the several forms peculiar to series D, in a number of samples of which it occurred, including some from a depth of more than 6 feet. The cells are isolated and spherical, 9–13 μ in diameter, reaching in exceptional cases 19 μ. There was usually a single bright green chloroplast, occasionally two, but neither pyrenoids nor starch were present, their place being taken by oil. Reproduction occurred by the formation of aplanospores which were produced in large numbers within a mother cell. These

small aplanospores gradually increase in size until they reach the dimensions of the ordinary vegetative cells. Until the swarmspores of the alga have been obtained, its systematic position cannot be stated.

Species H

In two cultures from a depth of 3 feet, brownish patches appeared between the sand and the glass at the bottom of the cultures, which were at first thought to be due to diatoms, but which proved, on examination, to consist of minute organisms probably of a flagellate nature. The tiny cells were 2.5–3 μ wide by 4.5–5 μ long, and had a bell-shaped brownish green chromatophore lining the greater part of the outer membrane. There was no pyrenoid. The organisms were not observed in the motile condition, and nothing is known of their cilia. There was always a very conspicuous projecting stigma, however, which indicates that they are normally motile.

Species I

This puzzling form occurred in only two cultures, one from a depth of 5 feet 8 inches and the other 8 feet 7 inches. It consists of small oval cells 4–7.5 μ long and 3–5 μ wide. There is a single parietal chloroplast which often does not cover the entire wall, and in which a conspicuous pyrenoid is embedded. Multiplication takes place by the formation of 2–16 aplanospores within a mother cell. Motile stages were not observed, and there is no tendency to the formation of gelatinous colonies.

Species J

? *Ankistrodesmus Pfitzeri* (Schröder) West, Brit. Freshw. Alg. 224. *f. 94 G, H.* 1904.

In association with the preceding species in culture 62 at a depth of 5 feet 8 inches were conspicuous elongated cells. In form they seemed to be very similar to *Ankistrodesmus Pfitzeri*, but they were somewhat smaller and perhaps also a little broader in proportion. The cells were about 10 μ long and 3 μ wide, and there was a parietal chloroplast but no pyrenoid. *A. Pfitzeri* is usually stated to occur in gelatinous colonies, but in this culture the cells, although tending to adhere to each other and therefore

being surrounded by some gelatinous material, did not occur in well-defined colonies. Unfortunately, the species did not persist for very long in the culture but was gradually crowded out by Species I.

Moss Protonema

Moss Protonema was observed in only one culture, at a depth of 6 inches. It can therefore be assumed that normally the filaments do not descend to any great depth as do some algae, but are comparatively superficial in their growth.

TABLE I

(Series A)

SAMPLES TAKEN NOVEMBER 18, 1922, BETWEEN THE EXPERIMENTAL GREENHOUSE AND THE OLD RESIDENCE

Number of culture	Depth	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Monocilia viridis*	*Stichococcus bacillaris*	*Stichococcus scopulinus*	*Protococcus*-like colonies	*Chlamydomonas* (unidentified)	Species A	Species B	Species D	Species E	Species F	*Nostoc muscorum*	*Nostoc commune*	*Nostoc* sp.	*Lyngbya subtilis*	*Phormidium* sp.	*Navicula atemoides*	*Navicula mutica*	*Hantzschia amphioxys*
½	6 inches	Apr. 1923	×	×						×										×××		×	×		
		May 1923								×															
		Nov. 1923	×									?											××	×	
		Apr. 1924	×										?	?			?	×	×				××	×	
1a	12 inches	Mar. 1923	×			×																			
		Apr. 1923	×			×																			
		Nov. 1923	×			×								×											
		Apr. 1924	×			×																			
1b	12 inches	Feb. 1923	×					×				?											××		
		Mar. 1923	×									?											×××		
		Apr. 1923	×				×					?													
		Nov. 1923	×	×		×						?	×			×							×××		
		Apr. 1924	×			×																	×		
2a	18 inches	Feb. 1923	×																						
		Mar. 1923	×																						
		Apr. 1923	×																						
		Apr. 1924	×									×													

TABLE I (*Continued*)

Number of culture	Depth	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Monocilia viridis*	*Stichococcus bacillaris*	*Stichococcus scopulinus*	*Protococcus*-like colonies	*Chlamydomonas* (unidentified)	Species A	Species B	Species D	Species E	Species F	*Nostoc muscorum*	*Nostoc commune*	*Nostoc* sp.	*Lyngbya subtilis*	*Phormidium* sp.	*Navicula atemoides*	*Navicula mutica*	*Hantzschia amphioxys*
2b	18 inches	Mar. 1923	×				×					×													
		Apr. 1923	×																						
		Nov. 1923	×																						
		Apr. 1924	×	×			×	×				×		×											
3	2 feet	Apr. 1923	×																						
		Nov. 1923	×																						
		Apr. 1924	×			×																			×
4	2 ft. 6 in.	Mar. 1923	×	×					?			×				×									
		Apr. 1923	×	×								×													
		Nov. 1923	×												×										
		Mar. 1924	×									×		×	×						×				
		Apr. 1924	×									×									×				
5	3 feet	Mar. 1923		×		×																			
		Apr. 1923		×																					
		Nov. 1923		×										×	×										
		Apr. 1924		×																					
6	3 ft. 6 in.	Feb. 1923		×				×																	
		Apr. 1923					×					×			×										
		Nov. 1923	×		×						×														
		Apr. 1924	×		×																				
7	4 feet	Feb. 1923			×							×													
		Apr. 1923	×																						
		Nov. 1923	×	×	×	×	×																		
		Apr. 1924	×	×		×																			
8	5 feet	Nov. 1923				×																			
		Apr. 1923				×																			

TABLE II

(Series B)

SAMPLES TAKEN APRIL 19, 1923, NORTHWEST OF CENTRAL LILY POND, MAIN ENTRANCE

Number of culture	Depth of sample	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botryidiopsis arhiza*	*Trochiscia reticularis*	*Ulothrix subtilis*	*Characiopsis minuta*	*Chlamydomonas* (unidentified)	Species A	Species B	Species D	Species E	*Protococcus*-like stage	*Gloeocystis*-like stage	*Nostoc* sp.	*Navicula atomoides*	*Navicula mutica*	*Nitzschia palea*	*Hantzschia amphioxys*
26a	1 ft.	May 1923	×		×						×											
	6 in.	Jan. 1924	×	?	×	×		×						×								
		Apr. 1924	×	×		×		×														
26b	1 ft.	May 1923	×		×						×											
	6 in.	Jan. 1924	×																			
		Apr. 1924	×	×				×														
29a	1 ft.	May 1923	×		×																	
	6 in.	Feb. 1924	×		×										×				×			
		Apr. 1924	×		×									×		×						
29b	1 ft.	May 1923	×		×						×											
	6 in.	Feb. 1924	×		×	×			×			×							×				
		Apr. 1924	×	?		×			×		×	×						×	×	×	×	
25a	2 ft.	May 1923	×								×											
	3 in.	Jan. 1924	×																			
		Apr. 1924	×	?		×													×			
25b	2 ft.	May 1923	×								×											
	3 in.	Jan. 1924	×	×																		
		Apr. 1924	×							×						×	×		×			
24a	2 ft.	May 1923	×		×						×											
	6 in.	Nov. 1923	×		×									×					×			×
		Mar. 1924	×	?	?	×																
		Apr. 1924		?	×	×										×			×			
24b	2 ft.	May 1923	×		×						×											
	6 in.	Dec. 1923	×		×														×			
		Apr. 1924	×											×					×			
27a	2 ft.	May 1923	×		×						×											
	6 in.	Jan. 1924	×		×									×					×			
		Apr. 1924	×	?	×																	
27b	2 ft.	May 1923	×		×																	
	6 in.	Jan. 1924	×	?	×						×											
		Apr. 1924	×	?	×									×								

TABLE II (*Continued*)

Number of culture	Depth of sample	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Ulothrix subtilis*	*Characiopsis minuta*	*Chlamydomonas* (unidentified)	Species A	Species B	Species D	Species E	*Protococcus*-like stage	*Gloeocystis*-like stage	*Nostoc* sp.	*Navicula atomoides*	*Navicula mutica*	*Nitzschia palea*	*Hantzschia amphioxys*
23a	3 feet	May 1923	×		×																	
		Nov. 1923	×	?	×	×					×								×			
		Apr. 1924	×	?	×	×					×								×			
23b	3 feet	May 1923	×		×																	
		Nov. 1923	×		×						×											
		Apr. 1924	×		×	×					×								×			
28a	3 feet	May 1923	×		×						×											
		Feb. 1924	×	×	×	×								×					×			
		Apr. 1924	×			×													×			
28b	3 feet	May 1923	×		×						×								×			
		Feb. 1924	×	?	×												×		×			
		Apr. 1924	×	?	×	×							×						×			
22a	3 ft. 6 in.	May 1923	×		×																	
		Nov. 1923	×	?	×						×											
		Apr. 1924	×			×							×									
22b	3 ft. 6 in.	May 1923	×																			
		Nov. 1923	×		×	×					×											
		Apr. 1924	×	?	×	×							×									
21a	4 feet	May 1923	×		×																	
		Nov. 1923	×	?	×	×					×											
		Apr. 1924	×	??	×	×										×						
21b	4 feet	May 1923	×	×	×																	
		Nov. 1923	×	?	×	×								×								
		Apr. 1924	×	?	×	×					×											
30	4 feet	May 1923	×		×						×											
		Feb. 1924				×									×				×			
		Apr. 1924	×		×	×	×										×		×			

TABLE III

(Series C)

SAMPLES TAKEN JUNE, 1923, SOUTH OF GRADUATE LABORATORY

Number of culture	Depth of sample	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Trochiscia* sp.	*Chlamydomonas* (unidentified)	*Dactylococcus* sp.	Species A	Species B	Species D	Species E	*Glococystis*-like stage	*Nostoc commune*	*Nostoc* sp.	*Phormidium tenue*	*Phormidium molle*	*Navicula atemoides*	*Nitzschia palea*
50	1 foot	Feb. 1924	×			×							×				×	×	×		×	
		Apr. 1924	×			×														×	×	×
49	1 ft. 4 in.	Oct. 1923	×		×																	
		Feb. 1924	×		×								×									
		Apr. 1924	×		×								×		×		×				×	
48a	1 ft. 8 in.	Oct. 1923	×		×					×												
		Feb. 1924	×	?	×					×												
		Apr. 1924	×	?	×	×							×		×							
48b	1 ft. 8 in.	Oct. 1923	×		×																	
		Feb. 1924	×		×																	
		Apr. 1924	×		×	×																
47	2 feet	Oct. 1923	×																			
		Feb. 1924	×	×								×										
		Apr. 1924	×	×						×		×										
46	2 ft. 3 in.	Oct. 1923	×		×			×														
		Feb. 1924	×		×			×		×												
		Apr. 1924	×		×			×													×	
45a	2 ft. 6 in.	Oct. 1923	×		×			×														
		Feb. 1924	×		×			×														
		Apr. 1924	×					×														
45b	2 ft. 6 in.	Oct. 1923	×		×																	
		Feb. 1924	×		×																	
		Apr. 1924	×								×			×								
44a	3 feet	Oct. 1923	×																			
		Feb. 1924	×	×						×												
		Apr. 1924	×	×						×		×									×	
44b	3 feet	Oct. 1923	×		×																	
		Feb. 1924	×		×																	
		Apr. 1924	×		×																	
43a	3 ft. 1 in.	Oct. 1923	×	?	×																	
		Feb. 1924	×	?	×	×																
		Apr. 1924	×	?	×	×																

TABLE III (*Continued*)

Number of culture	Depth of sample	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Trochiscia* sp.	*Chlamydomonas* (unidentified)	*Dactylococcus* sp.	Species A	Species B	Species D	Species E	*Gloeocystis*-like stage	*Nostoc commune*	*Nostoc* sp.	*Phormidium tenue*	*Phormidium molle*	*Navicula atemoides*	*Nitzschia palea*
43b	3 ft. 1 in.	Oct. 1923	×			×																
		Feb. 1924	×			×																
		Apr. 1924	×			×							×									
43c	3 ft. 1 in.	Oct. 1923					×															
		Feb. 1924					×															
		Apr. 1924					×															
42a	3 ft. 3 in.	Oct. 1923				×																
		Jan. 1924				×							×									
		Apr. 1924				×							×									
42b	3 ft. 3 in.	Oct. 1923	×																			
		Feb. 1924	×	?												×						
		Apr. 1924	×	×		×						×									×	
42c	3 ft. 3 in.	Oct. 1923																				
		Feb. 1924										×										
		Apr. 1924										×										
41a	3 ft. 6 in.	Oct. 1923	×																			
		Feb. 1924	×										×	×								
		Apr. 1924	×										×									
41b	3 ft. 6 in.	Oct. 1923	×																			
		Feb. 1924	×																		×	
		Apr. 1924	×																		×	
41c	3 ft. 6 in.	Oct. 1923	×																			
		Feb. 1924											×									
		Apr. 1924											×									
40a	3 ft. 9 in.	Produced no growth																				
40b	3 ft. 9 in.	Oct. 1923	×																			
		Feb. 1924	×					×														
		Apr. 1924	×																			
40c	3 ft. 9 in.	Oct. 1923	×																			
		Feb. 1924	×			×				×												
		Apr. 1924	×			×		×		×												

TABLE IV

(Series D)

SAMPLES TAKEN IN "LOWER" GARDEN BEHIND GREENHOUSES, JANUARY 14, 1924. (TEMP. 22° F.)

Number of culture	Depth of sample	Weight of inoculum	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Stichococcus bacillaris*	*S. scopulinus*	*Ulothrix subtilis*	*Uronema confervicola*	Species B	Species C	Species E	Species F	Species G	Species H	Species I	Species J	Moss Protonema	*Nostoc comminutum*	*Oscillatoria amphibia*	*Navicula atemotdes*	*Nitzschia palea*
77	6 in.	5 gm.	Feb. 1924	×	×				×		×					×						×				
			Apr. 1924	×	×				×		×	×														
76i	12 in.	.01 gm.	Mar. 1924	×					×																	
			Apr. 1924	×					×																	
76h	12 in.	.02 gm.	Mar. 1924	×					×																	
			Apr. 1924	×					×																	
76g	12 in.	.05 gm.	Mar. 1924	×					×					×												
			Apr. 1924	×					×																	
76a	12 in.	.1 gm.	Feb. 1924	×				×	×																×	
			Apr. 1924	×				×	×																	
76b	12 in.	.2 gm.	Feb. 1924	×				×	×					×												
			Apr. 1924	×				×	×																	
76c	12 in.	.5 gm.	Feb. 1924	×					×					×												
			Apr. 1924	×					×																	
76d	12 in.	1 gm.	Feb. 1924	×				×						×												
			Apr. 1924					×	×					×												
76e	12 in.	2 gm.	Feb. 1924	×			×	×	×		×			×												
			Apr. 1924	×				×	×		×			×												
76f	12 in.	5 gm.	Feb. 1924	×			×	×		×							×	×							×	
			Apr. 1924	×				×			×						×	×								
75	1 ft. 6 in.	5 gm.	Mar. 1924	×				×	×				×	×												
			Apr. 1924	×				×	×				×													
74a	2 feet	.1 gm.	Mar. 1924																×							
			Apr. 1924																×							
74b	2 feet	.2 gm.	Mar. 1924	×																						
			Apr. 1924	×				×																		
74c	2 feet	.5 gm.	Mar. 1924	×	×									?												
			Apr. 1924	×																						
74d	2 feet	1 gm.	Mar. 1924	×				×		×									×							
			Apr. 1924	×				×		×																
74e	2 feet	2 gm.	Mar. 1924	×						×				×												×
			Apr. 1924	×										×												
74f	2 feet	5 gm.	Mar. 1924	×	×	×	×	×		×				×		×										
			Apr. 1924	×	×			×		×																

TABLE IV (*Continued*)

Number of culture	Depth of sample	Weight of inoculum	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Stichococcus bacillaris*	*S. scopulinus*	*Ulothrix subtilis*	*Uronema confervicola*	Species B	Species C	Species E	Species F	Species G	Species H	Species I	Species J	Moss Protonema	*Nostoc commune*	*Oscillatoria amphibia*	*Navicula atomoides*	*Nitzschia palea*
73	2 ft. 6 in.	5 gm.	Mar. 1924 Apr. 1924	X X			X X		X X	X				X			X								X	
72	2 ft. 9 in.	5 gm.	Mar. 1924 Apr. 1924	X X		X X	X X	X	X X																X X	
71a	3 feet	.1 gm.	Mar. 1924 Apr. 1924	X										X X												
71b	3 feet	.2 gm.	Mar. 1924 Apr. 1924												X X											
71c	3 feet	.5 gm.	Mar. 1294 Apr. 1924	X X										X X												
71d	3 feet	1 gm.	Mar. 1924 Apr. 1924				X X		X			X	X	X X					X							
71e	3 feet	2 gm.	Mar. 1924 Apr. 1924	X X	X								X	X X		X X			X							
71f	3 feet	5 gm.	Mar. 1924 Apr. 1924	X X	X		X		X			X X		X X		X										
70a	3 ft. 6 in.	5 gm.	Mar. 1924 Apr. 1924																				X X			
70b	3 ft. 6 in.	over 5 gm.	Mar. 1924 Apr. 1924	X X	X X	X		X							? ?											
69a, b, c	4 ft. 2 in.	.5 gm., 1 gm., and 2 gm. respectively		Produced no growth																						
69d	4 ft. 2 in.	5 gm.	Mar. 1924 Apr. 1924															X X								
69e	4 ft. 2 in.	over 5 gm.	Mar. 1924 Apr. 1924	X X										X X				X								X
68a, b	5 ft. 5 in.	.5 gm. and 1 gm. resp.	July 1924	X																						
68c	5 ft. 5 in.	2 gm.	Mar. 1924 Apr. 1924																						X X	
68d	5 ft. 5 in.	5 gm.	Mar. 1924 Apr. 1924	X ?																						

TABLE IV (*Continued*)

Number of culture	Depth of sample	Weight of inoculum	Date of examination	*Chlorococcum humicola*	*Chlorochytrium paradoxum*	*Protosiphon botryoides*	*Chlorella* sp.	*Botrydiopsis arhiza*	*Trochiscia reticularis*	*Stichococcus bacillaris*	*S. scopulinus*	*Ulothrix subtilis*	*Uronema confervicola*	Species B	Species C	Species E	Species F	Species G	Species H	Species I	Species J	Moss Protonema	*Nostoc commune*	*Oscillatoria amphibia*	*Navicula atemoides*	*Nitzschia palea*
68e	5 ft. 5 in.	over 5 gm.	Mar. 1924 Apr. 1924	X X										X												
67a	5 ft. 8 in.	.5 gm.	May 7 1924																	X						
67b-c	5 ft. 8 in.	1 gm., 2 gm., 5 gm. and over, respectively		Produced no growth																						
66	6 ft. 4 in.	over 5 gm.		Produced no growth																						
65a-c	6 ft. 10 in.	.5 gm., 1 gm., and 2 gm. respectively		Produced no growth																						
65d	6 ft. 10 in.	5 gm.	Mar. 1924 Apr. 1924															X X								
65e	6 ft. 10 in.	over 5 gm.		Produced no growth																						
64	7 ft. 6 in.	over 5 gm.		Produced no growth																						
63a-d	8 ft. 2 in.	.5 gm., 1 gm., 2 gm. and 5 gm. respectively		Produced no growth																						
63e	8 ft. 2 in.	over 5 gm.	Apr. 1924																					X		
62	8 ft. 7 in.	over 5 gm.	Apr. 1924																	X	X					
61a-c	9 ft. 1 in.	1 gm., 2 gm., 5 gm. respectively		Produced no growth																						
61d	9 ft. 1 in.	over 5 gm.	Mar. 1924											X												
60a-c	9 ft. 5 in.	1 gm., 2 gm., and 5 gm. respectively.		Produced no growth																						

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PSEUDO-FERTILITY IN NICOTIANA[1]

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INTRODUCTION

It has long been known that the eggs of certain hermaphroditic plants and animals cannot usually be fertilized by pollen or sperms from the same individual, and this phenomenon has been termed self-sterility. Although it has been demonstrated in only one animal, *Ciona intestinalis*, the phenomenon has been shown to be fairly widespread throughout the plant kingdom. In 1895, Knuth listed 134 observed self-sterile species of Angiosperms belonging to 46 families, and East and Park ('17) estimated that 70 per cent of these observations proved definitely that the species were self-sterile. Hence, as early as 1895, at least 100 self-sterile species of Angiosperms had been observed.

In *Nicotiana*, the primary difference between sterile and fertile combinations has been shown (East and Park, '18) to be the difference in rate of pollen-tube growth. In incompatible combinations, the pollen grains germinate but do not grow with sufficient rapidity to reach the ovary before the flower falls. They showed, through cytological studies of styles taken at successive twelve-hour intervals after pollination, that the pollen-tubes of fertile combinations exhibit an acceleration in their rate of growth, their growth curves when plotted being similar to those representing autocatalytic reactions. On the other hand, the pollen-tubes of sterile combinations maintain a constant rate of growth, showing no acceleration, their growth curves being straight lines. By virtue of the acceleration in the rate of growth, the pollen-tubes of compatible matings reach the ovary in less than 96 hours after pollination, whereas in incompatible matings they fail to reach the ovary within the life of the flower.

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of master of science in the Henry Shaw School of Botany of Washington University.

Cross-incompatibility among self-sterile individuals was not described in the early work on self-sterility, and it was not until the work of de Vries ('06) on the self-sterile species *Linaria vulgaris* that the existence of intra-sterile, inter-fertile classes was first observed. That is, de Vries demonstrated the fact that there were among the self-sterile individuals of *Linaria vulgaris* with which he was working, two classes, and that every individual belonging to one class was cross-sterile with every individual of the same class and cross-fertile with every individual of the other class. Since that time, such intra-sterile, inter-fertile classes have been observed in various species by Correns ('12, '16), East and Park ('17), Baur ('19), Lehmann ('19), Crane ('23), Shull ('23), and Anderson ('24). East and Park ('17) showed that this cross-sterility is identical in nature with self-sterility, and that an interpretation which holds for one phenomenon must likewise hold for the other.

However, among self-sterile plants, individuals exhibiting self-fertility to some degree are frequently found. East and Park ('17) occasionally obtained, in their self-sterile hybrids between *Nicotiana alata* and *N. Forgetiana* and in several self-sterile species of *Nicotiana*, small capsules which contained relatively few seeds and rarely, if ever, approximated the size of those resulting from fertile combinations. It has been agreed by all investigators of the subject that a plant is self-fertile if it sets a full capsule of seeds when pollinated with its own pollen, and self-sterile if no seeds are set after self-pollinations. Neither of these two categories, however, includes those individuals of self-sterile species which exhibit a small but varying amount of self-fertility, and such plants have been described by East and Park as being "pseudofertile."

This phenomenon has been described, although in some cases not by this name, by Correns ('12, '13, '16) in the self-sterile species *Cardamine pratensis* and *Linaria vulgaris;* East and Park ('17, '18) in several species of *Nicotiana* and in hybrids between *Nicotiana alata* and *N. Forgetiana;* Sutton ('18) in plums and cherries; Baur ('19) in *Antirrhinum hispanicum;* Lehmann ('19) in *Veronica syriaca;* Shull ('23) in *Bursa grandiflora;* Anderson ('24) in *Nicotiana alata, N. Forgetiana,* and hybrids between *N.*

alata and *N. Forgetiana;* and Smith ('24) in hybrids between *N. alata* and *N. Forgetiana.*

East and Park ('17) observed the occurrence of pseudo-fertile individuals particularly among plants which were nearing the end of the flowering season, and hence exhibiting weakened vigor. By comparing the curve of pollen-tube growth in a pseudo-fertile combination with that in a self-sterile mating, East and Park ('18) found indication of the possibility that the so-called phenomenon of pseudo-fertility was a variant of self-sterility which was brought about by unfavorable environmental conditions and general decrease in the vigor of the plant. That is, there was apparently little or no acceleration in the rate of growth of the pollen-tubes such as is found in normal fertile combinations, but the growth was merely more rapid throughout the course of the pollen-tube through the style.

Stout ('16, '17), in connection with his studies on self- and cross-pollinations in *Cichorium Intybus*, observed a considerable variation in the expression of self-sterility and attributed it to germinal disturbances, which, however, he concluded were too variable to permit of any type of Mendelian interpretation. This feeble or "partial compatibility," as he terms it, he believes "manifests itself quite indiscriminately throughout the entire period of bloom" and is independent of any decrease in vegetative vigor. Hence, according to him, end-season fertility is comparatively rare and is not a condition commonly operating in incompatible plants. It is quite possible that he was observing in *Cichorium Intybus* the same phenomenon which was termed "pseudo-fertility" by East and Park ('17) in their work on *Nicotiana* hybrids, and that he has failed to distinguish between pseudo-fertility and true self-fertility. That is, it is possible that the "sporadic development of self-compatibility giving self-fertility among the progeny of self-sterile lines of descent" which he observed may be due to other changes rather than germinal disturbances. However, it is true that if the variations which he finds among self-sterile individuals are expressions of pseudo-fertility rather than of true fertility, the degree of pseudo-fertility must be much higher and much more variable in *Cichorium Intybus* than it is in *Nicotiana alata, N. Forgetiana,* and hybrids between them.

Stout ('20) reported results of continued work on *Cichorium Intybus* and of preliminary work on other genera. In *Cichorium Intybus*, he failed to find any progressive seasonal increase in pseudo-fertility. In *Brassica pekinensis* he observed a few cases of mid-seasonal fertility. *Eschscholtzia californica* was found to be somewhat pseudo-fertile, one plant in 200 setting a capsule comparable to those resulting from fertile pollinations, whereas *Raphanus sativus* was still more self-sterile, only one plant in 200 showing any indication of pseudo-fertility.

Sutton ('18) concludes that in plums and cherries the results are "consistent with the supposition that the plants consist of two larger classes, self-fertiles and self-steriles, with a smaller number of plants of intermediate properties." For this smaller group, she offers two possible interpretations. The intermediate group and some of the self-fertiles may be supposed to be heterozygous, and the self-steriles, homozygous, the occasional indications of partial self-fertility among the latter being attributable to errors probably. On the other hand, when a few fruits are formed out of a large number of pollinated flowers, the fact may mean that compatibility exists in a very slight degree. "If this could be confidently asserted," she adds, "it would be tempting to suppose that the tree may be a mosaic in that respect."

It is this phenomenon of pseudo-fertility, defined by East and Park ('17) and described by numerous investigators before and since, with which the present paper is concerned. In the course of this investigation on *Nicotiana alata* and on hybrids between *N. alata* and *N. Forgetiana*, a comparative measure of pseudo-fertility has been developed, by means of which it has been possible to demonstrate that pseudo-fertility in self-sterile matings is of the same order as that in cross-sterile matings, that pseudo-fertility is of a different order from true fertility but of the same order as self-sterility, and that in genetic strains concerned, the phenomenon was but slightly affected by environmental changes, or with progress of the flowering season. Measurements of the pollen grains were made and indications were found that the percentage of variability in diameter is significantly greater in plants resulting from self-pollinations than in those coming from cross-pollinations.

Materials and Methods

In the present series of investigations on the phenomenon of pseudo-fertility, *Nicotiana alata* Lk. and Otto var. *grandiflora* Comes, and F_7 and F_8 hybrids between *N. alata* and *N. Forgetiana* were used, the seed coming originally from stock used in investigations on self-sterility at the Bussey Institution of Harvard University (see East, '15; East and Park, '17; and Anderson, '24). Strains of the pure species and of the hybrids coming from seeds planted in September, 1922, were taken over by the author in January, 1923, and during the next 18 months the following investigations were conducted on that and the succeeding generation of plants.

The material was particularly satisfactory for such a series of investigations. Capsules containing, on an average, between 300 and 500 seeds each were set in 95 per cent of the compatible combinations; and thus pseudo-fertility, in which usually only a few seeds are set at more or less infrequent intervals, was readily distinguished from true fertility. The plants grow well under greenhouse conditions, and they can be cut back and made to pass through a second flowering season, if so desired. However, due to the attacks of mosaic and to the extremely hot summers, it is almost impossible to carry the plants over from one season to the following in this climate.

Experimental error due to the contamination of pollen or to accidental pollination of flowers was reduced as far as possible. With the anthesis of the first few flowers of a plant, the panicle was enclosed in a paper bag so as to prevent contamination or cross-pollination by wind or insects. When the plants were unbagged for the purpose of pollinating the flowers, care was taken that pollen from one plant was not permitted to fall on the flowers of near-by plants. In making pollinations, newly opened flowers were used in order that there might be as little danger as possible of contamination by foreign pollen. The pollinations were effected by carefully dusting the stigma of a given flower with the desired pollen. When necessary, the flowers were emasculated before being pollinated. Also, after each emasculation or pollination the hands and forceps were washed in 95 per cent alcohol in order to kill all the pollen. That this procedure

was effective in the prevention of contamination by foreign pollen was demonstrated by Anderson ('24), as follows: The fingers were dusted with pollen and 4 pollinations made. Then, after rinsing the hands with alcohol, 4 more pollinations were made on the same plant with the remaining pollen. Full capsules were set in the first 4 pollinations and none in the last, thus showing that the alcohol was efficient in destroying the pollen grains.

PRESENTATION OF DATA AND DISCUSSION

SELF-STERILITY, SELF-FERTILITY, AND PSEUDO-FERTILITY

As has already been stated, the object of these investigations has been a study of pseudo-fertility, particularly with respect to the relation which it bears to self- and cross-sterility, and cross-fertility. It has been indicated by East and Park ('18), through a study of the relative rates of pollen-tube growth, that pseudo-fertility in *Nicotiana* is of the nature of true sterility rather than of that of true fertility. That is, their data indicated that the phenomenon was probably a variant of true sterility, brought about by unfavorable environmental conditions, and not a modified expression of true fertility resulting from germinal modifications in a self-sterile race. The present series of investigations was designed to prove definitely, by a method other than that of pollen-tube growth, that, in *Nicotiana*, pseudo-fertility is of the order of self-sterility and not of self-fertility.

The method used centers about the fact which had previously been observed by Anderson that pollinated unopened buds will frequently exhibit pseudo-fertility when mature flowers will not. With this idea in mind, series of pollinations were made simultaneously on unopened buds, and first and second flowers of the same branch of the panicle (fig. 1). The flowers were numbered from apex to base of the branch of the panicle, thus making the first the youngest of the mature flowers. The series of pollinations were made and the panicles bagged. After one week, those flowers, the ovaries of which showed no enlargement or indication of setting seed, were removed. This was justified by the previously determined fact that certain indication of capsule formation appears within the first week after pollination. Those flowers which indicated that seeds were being set were allowed to

remain on the plant until the capsule showed signs of dehiscence, at which time they were removed. When thoroughly dry, the seeds in each capsule were counted and recorded. As there was not sufficient time to allow those capsules formed during the latter part of the work to mature on the plant, they were removed two

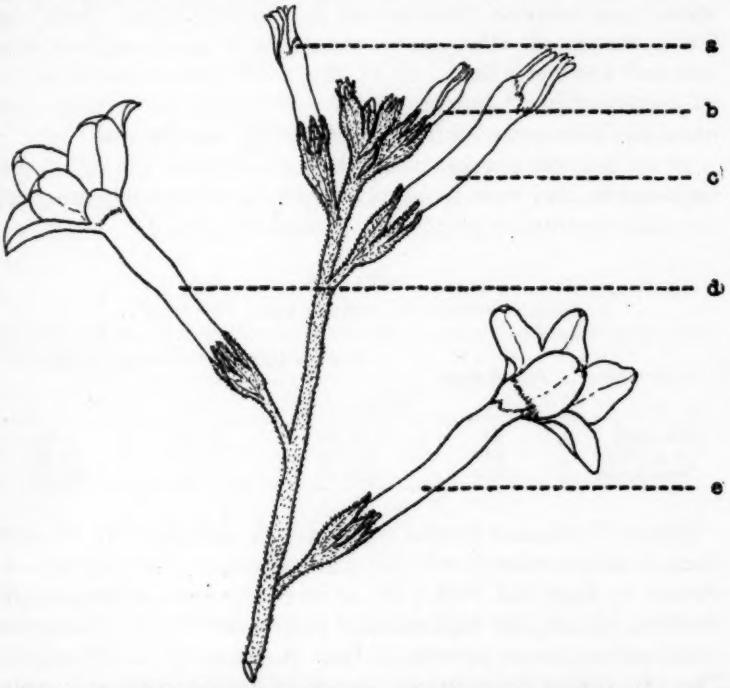


Fig. 1. Typical branch of a panicle: a, young bud; b, very young bud; c, unopened bud; d, first flower; e, second flower.

weeks after the pollinations were made and were dried in an oven at 100° C. for 30 minutes. This heating served to dry the seeds so that they could be readily separated and counted, and was possible by virtue of the fact that the chief aim was a determination of the number of seeds set, and that the seeds were not needed for the plants of the next season.

Such series of pollinations were made on both the hybrids and the pure species, *Nicotiana alata*, but at this point only those

results obtained from the hybrids are of interest. Two hundred thirty-three series, including self-sterile, cross-sterile, and cross-fertile combinations were made on the hybrid plants. For the resulting data see "Complete Data" at the close of this paper. In several instances, capsules were formed and were lost before the seeds were counted (represented in the "Complete Data" by "?"), but in all other cases, the seeds of every capsule were counted and recorded. All of the series are included in the "Complete Data," but any series containing one or more such cases has been excluded from the following calculations.

In table 1, the average number of seeds set in the self-sterile, cross-sterile, and cross-fertile combinations respectively are given and these results are graphically presented in fig. 2.

TABLE I

PSEUDO-FERTILITY VERSUS TRUE FERTILITY

Pollinations	No. of series	Average number of seeds per capsule		
		Unopened bud	1st flower	2nd flower
Self-sterile	84	86.36	27.92	5.63
Cross-sterile	63	138.65	32.16	18.88
Cross-fertile	41	439.46	465.83	414.80

Figure 2 expresses several fundamental relations. In the first place, it shows quite clearly that just as cross-sterility was demonstrated by East and Park ('17) to be of the same nature as self-sterility, so here, the expression of pseudo-fertility in cross-sterile combinations is comparable to that in self-sterile combinations. The two curves representing pseudo-fertility in self- and cross-sterile combinations are essentially the same and are in relatively the same position on the graph. The only interpretation which can be applied to this close similarity between these two curves is that pseudo-fertility in cross-sterile combinations is of the same order as that in self-sterile pollinations.

The second and probably the most fundamental relation to be obtained from table 1 and the curves in fig. 2 is that pseudo-fertility is not of the nature of true fertility. The curve representing true fertility is not only of a different type from those representing pseudo-fertility, but it occupies an area on the

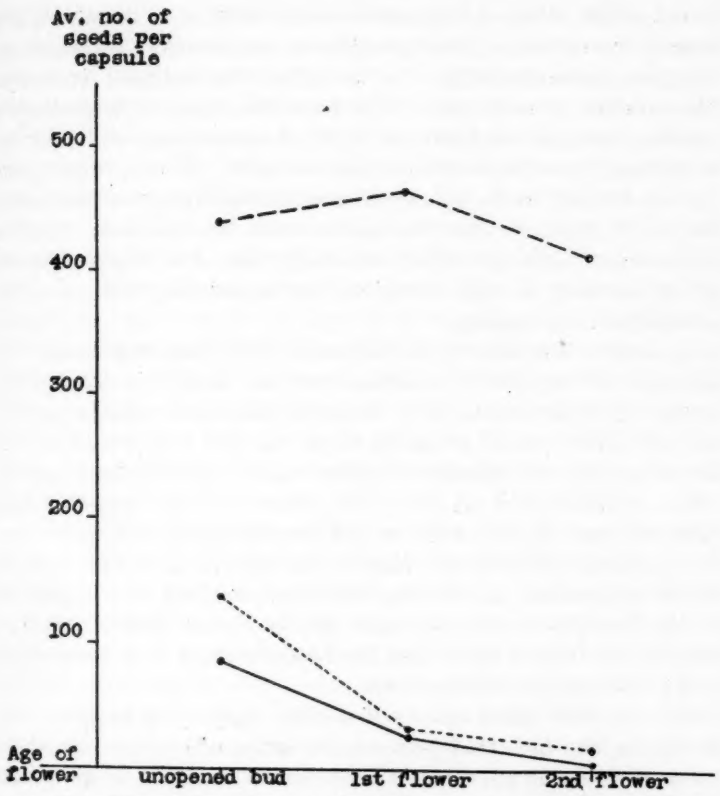


Fig. 2. Pseudo-fertility versus true fertility:———, self-sterile pollinations; ·····, cross-sterile pollinations; — — — —, cross-fertile pollinations.

graph which is widely separated from that occupied by the curves representing pseudo-fertility. In effect, the lowest point on the curve of the former is practically three times as high as the highest point of either of the latter two curves. It is indeed quite obvious that pseudo-fertility as expressed in the hybrids between *Nicotiana alata* and *N. Forgetiana* cannot be of the same order as true fertility.

Just as it is obvious even from a casual examination of fig. 2 that pseudo-fertility in either self- or cross-sterile combinations

is not of the order of complete compatibility, it is likewise quite clearly shown that it is comparable to, and probably a variant of, complete incompatibility. In the graph, the ordinate represents the number of seeds set. Therefore, the curve of truly sterile combinations, or combinations in which no seeds are set, would be a straight line coincident with the abscissa. Hence, to compare pseudo-fertility with complete incompatibility, it is necessary merely to compare the two curves with the abscissa. Such a comparison indicates rather decidedly that the relationship of pseudo-fertility is with complete incompatibility and not with complete compatibility.

A study of the curves themselves is somewhat significant. In the case of truly fertile combinations, one would expect pollinations of first flowers to yield the most abundant seeds, since the second flowers would probably be so old that they would wither before a sufficient number of pollen-tubes reached the ovary to effect fertilization of all the ovules, whereas in the unopened bud probably not all the ovules would be sufficiently well developed to permit of fertilization. Hence, one would expect the curve of fertile pollinations to rise from the unopened bud to a maximum in the first flower and fall again in the second flower. This is exactly the type of curve that has been obtained from an average of 41 cross-fertile combinations.

On the other hand, since self-sterility (according to East, '15) is due to the fact that pollen-tubes after self-pollination show no acceleration in growth and hence fail to reach the ovary within the life of the flower, it would be expected that pollinations of unopened buds would yield the most seeds by virtue of the fact that in such pollinations additional time is gained and the pollen-tubes may reach the ovary before the flower falls. That this is true, is quite evident in the curve which shows a sudden drop from the unopened bud to the first flower followed by a gradual decline to the second flower. This is further evidence that the pseudo-fertility of self- and cross-sterile combinations is not of the same order as true fertility.

In this connection, observations made on a single plant, WA-1, are of interest. This plant was a *Nicotiana alata* × *N. Forgetiana* hybrid of a genetic strain other than that to which the remainder

of the plants used belonged and hence not included in the "Complete Data." It was apparently completely self-sterile, no seeds being set in 17 series of pollinations made on unopened buds, first and second flowers. Series were pollinated at intervals throughout the flowering season of the plant, which, in this case, extended over a period of 50 days, and there was no evidence whatsoever of an end-seasonal pseudo-fertility. It was, however, possible to obtain seeds in considerable numbers by pollinating buds from 4 to 6 days before anthesis, as shown in table II. Hartley ('02), in attempting premature fertile pollinations in *Nicotiana Tabacum*, was not only unable to get the flowers to set seed, but also found that the growth of the pollen-tubes into the ovaries before the ovules were sufficiently mature for fertilization resulted in an injury which caused the flowers to fall immediately. In view of his observations, it is interesting that in these sterile combinations, seeds can be set in considerable numbers by pollinating the very young buds.

In the light of this comparison between the curves representing true fertility and the degree of pseudo-fertility as indicated by the number of seeds set in the unopened bud, first and second flowers, we might consider the number of seeds set in younger buds and older flowers. In cross-fertile combinations seeds have been set in the third and fourth flowers in the few cases where they have been tried, but always fewer than in the second flower. Therefore, as the flower ages, fewer and fewer seeds are set until theoretically we reach an age at which no seeds are set. At this place, the curves representing true fertility and pseudo-fertility would be coincident with each other and with the abscissa. Likewise, we can conceive of a point at the opposite end of the curves where they would pass through the same point. As younger and younger buds are pollinated in sterile combinations, more and more seeds are set and the curve for pseudo-fertility rises. As will be shown in the case of WA-1, plants which are apparently completely self-sterile when pollinated in the unopened bud, first and second flowers may be pseudo-fertile to a considerable degree if pollinations on younger buds are made. However, in cross-fertile matings, fewer seeds were set in unopened buds than in the first flowers. As younger buds would be pollinated, it seems probable

that fewer and fewer seeds would be set. In effect, Hartley ('02) found in *Nicotiana Tabacum* that very early fertile pollinations caused the flowers to fall before any seeds were set. Hence, we can conceive of the curve for true fertility falling off rather abruptly, and in the drop it would probably cross the rising curve for pseudo-fertility. Therefore, if the two curves would be carried out sufficiently far in either direction it is very probable that they would meet. This point should be considered in any attempt to distinguish between pseudo-fertility and true fertility, and the phenomena should be studied on flowers at that age at which the difference is greatest.

TABLE II

PSEUDO-FERTILITY IN PREMATURE POLLINATIONS

Pollination	Date	No. of seeds per capsule		
		Very young bud	Young bud	Unopened bud
WA-1 selfed	10-24-23	0	0	0
WA-1 selfed	10-24-23	150+	0	0
WA-1 selfed	10-24-23	37	0	0
WA-1 selfed	10-24-23	0	0	0
WA-1 selfed	10-24-23	432	0	0
WA-1 selfed	11- 3-23	319	0	0
WA-1 selfed	11- 3-23	352	0	0
WA-1 selfed	11- 3-23	487	0	0
WA-1 selfed	11-12-23	0	6	0
WA-1 selfed	11-19-23	0	454	0
WA-1 selfed	11-19-23	440	0	0
WA-1 selfed	11-24-23	0	391	0
WA-1 selfed	11-24-23	167	0	0
WA-1 selfed	12- 7-23	0	0	0
WA-1 selfed	12- 7-23	0	150	0
WA-1 selfed	12-13-23	0	0	0

In the table, buds before anthesis are termed young buds and those 6 days before anthesis are termed very young buds. It is of interest to find that seeds can be set by pollinations made so early in the bud, and the fact may prove to be of practical significance in obtaining seeds from normally incompatible combinations. However, before its practical significance could be asserted, it would be necessary not only to run germination tests on the seeds produced by such early pollinations to see whether or not they are fertile, but to discover the extent to which it is possible to obtain seeds by this means from other Angiosperms.

As has been said earlier, the panicles were placed in paper bags so as to avoid cross-pollination by wind or insects. In view of the fact that environmental factors have been thought to increase pseudo-fertility, the question arose as to whether the bagging of the panicles in any way influenced the degree of pseudo-fertility expressed by the plant. In order to determine any such possible influence, the following experiment was carried out.

Two series were made simultaneously on different branches of the same panicle of the plant. The branch bearing one series was bagged, whereas that bearing the other was not included. To avoid accidental cross-pollination in the case of those not bagged, the corollas of the flowers were tied, and in that way no foreign pollen could reach the stigma. Ten such series, including both self- and cross-sterile combinations, were made on different plants, the data of which are given in table III.

TABLE III

INFLUENCE OF BAGGING ON PSEUDO-FERTILITY

Pollination	Date	Number of seeds per capsule					
		Unopened bud		1st flower		2nd flower	
		Bagged	Unbagged	Bagged	Unbagged	Bagged	Unbagged
CA–4×CB–13	4–19–23	338	158	0	0	85	0
CC–1×CC–5	4–26–23	355	461	390	381	338	261
CC–5×CC–1	4–26–23	0*	0	0	?	0	?
CB–4×CG–8	4–19–23	230	292	87	105	41	0
CG–20×CL–4	4–19–23	339	0	0	0	0	0
CC–1 selfed	4–26–23	0	0	0	0	0	0
CC–5 selfed	4–26–23	0	0	0	0	0	0
WA–1 selfed	10–19–23	0	0	0	0	0	0
WA–1×WB–1	10–19–23	0	0	0	0	0	0
WB–1 selfed	10–20–23	0	0	0	0	0	0
Average		140.2	101.22	53	54	52.6	29

* This series not included in the averages because of the lost capsules in the unbagged first and second flowers.

A comparison of the average number of seeds set in the bagged series with that in the unbagged, together with a comparison of their respective graphs (fig. 3), shows quite clearly that bagging does increase the degree of pseudo-fertility. Considering together the average number of seeds set in unopened bud, first and second flowers, there has been an increase of 33.42 per cent in the

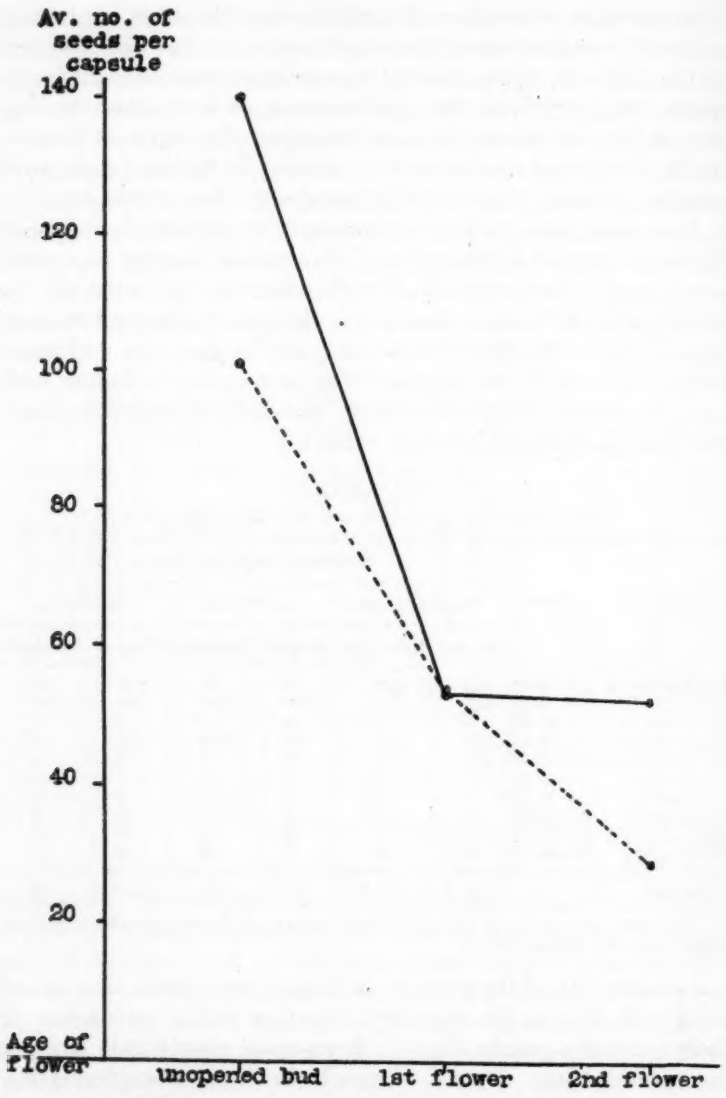


Fig. 3. Influence of bagging on pseudo-fertility: ———, bagged flowers; ....., unbagged flowers.

number of seeds set in the bagged over those in the unbagged panicles. The fact that pseudo-fertility is thus increased by bagging must be remembered in considering the actual values of the data as given. However, bagging was practised throughout the entire series of pollinations and hence the relative values still remain true. The error resulting from bagging the flowers is constant throughout the experiments and hence does not affect the conclusions which are to be drawn from the data recorded.

INFLUENCE OF SEASONAL PROGRESS

East and Park ('17) suggested that pseudo-fertility was a variant of self-sterility brought about by unfavorable environmental conditions which caused a decrease in the vigor of the plant. Particularly did they find pseudo-fertility near the end of the flowering season. With this idea in mind, successive series of self-pollinations were made on 16 individuals during the early, and then during the latter part of their flowering season. The flowering season of the plants grown in the Bussey Institution was sometimes as long as 3 months, but in our greenhouses this was not the case. Only rarely did the flowering season of any one plant continue for more than 3 weeks, and usually it lasted only 10 days or 2 weeks. Hence, with the exception of the plant WA-1 previously cited, the interim between the early and later pollinations was a week to 18 days. The data from these repeated series are given in table IV.

TABLE IV

SEASONAL CHANGE IN PSEUDO-FERTILITY

Pollination	Date	Number of seeds per capsule		
		Unopened bud	1st flower	2nd flower
GOB-4 selfed	4-21-24	0	0	0
GOB-4 selfed	4-30-24	314	0	0
GA-2 selfed	4-18-24	0	0	0
GA-2 selfed	4-30-24	27	0	0
GC-3 selfed	4-14-24	124	0	0
GC-3 selfed	4-26-24	387	0	0
GC-6 selfed	4-18-24	0	0	0
GC-6 selfed	4-26-24	149	0	0

TABLE IV (*Continued*)

Pollination	Date	Number of seeds per capsule		
		Unopened bud	1st flower	2nd flower
GF-1 selfed	4-16-24	0	0	0
GF-1 selfed	4-28-24	329	0	0
GF-1 selfed	4-30-24	0	0	0
LH-1 selfed	4-21-24	67	0	0
LH-1 selfed	4-28-24	45	79	0
CB-14 selfed	3-27-23	473	136	48
CB-14 selfed	4-14-23	550	29	54
GC-1 selfed	3-29-24	48	0	0
GC-1 selfed	4- 8-24	14	0	0
GF-2 selfed	4-16-24	121	0	0
GF-2 selfed	4-28-24	0	0	0
GF-9 selfed	4-21-24	0	208	0
GF-9 selfed	4-28-24	0	0	0
GOA-2 selfed	4-16-24	419	154	0
GOA-2 selfed	4-30-24	7	0	0
GOF-3 selfed	4-18-24	0	0	0
GOF-3 selfed	4-30-24	0	0	0
GE-1 selfed	4-21-24	0	0	0
GE-1 selfed	4-28-24	0	0	0
GOA-1 selfed	4-11-24	0	0	0
GOA-1 selfed	4-30-24	0	0	0
GOA-3 selfed	4-18-24	0	0	0
GOA-3 selfed	4-30-24	0	0	0
GOA-4 selfed	4-21-24	0	0	0
GOA-4 selfed	4-30-24	0	0	0

The possible change in degree of pseudo-fertility with seasonal progress is best studied by comparing averages (see table v) of the number of seeds set in the early part of the season with those in the later pollinations. From this table and fig. 4 it appears evident

TABLE V

SEASONAL CHANGE IN PSEUDO-FERTILITY

	Average number of seeds per capsule		
	Unopened bud	1st flower	2nd flower
Early pollinations	78.25	31.125	3
Later pollinations	107.625	6.75	3.375

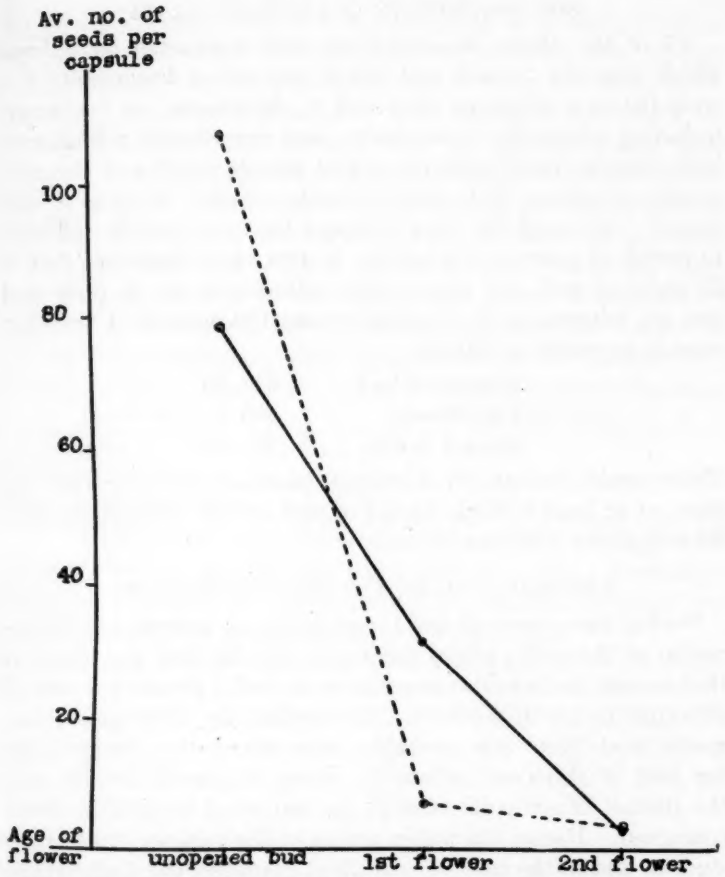


Fig. 4. Seasonal change in pseudo-fertility: ———, early pollinations; ....., later pollinations.

that, considering all 3 pollinations of each series, there was very little change of pseudo-fertility with the progress of the season. The average number of seeds set in the later series is only 4.78 per cent greater than that in the early pollinations and this is scarcely great enough to be significant. Hence, there was little if any seasonal change in pseudo-fertility shown in the *Nicotiana alata* × *N. Forgetiana* hybrids used in the present investigations.

PSEUDO-FERTILITY IN NICOTIANA ALATA

All of the above investigations were conducted on hybrids which were the seventh and eighth generation descendants of a cross between *Nicotiana alata* and *N. Forgetiana*. A few series, including self-sterile, cross-sterile, and cross-fertile pollinations, have likewise been made on several genetic families of the pure species, *Nicotiana alata* (see "Complete Data" at close of this paper). Although the data obtained here may not be sufficient to permit of positive conclusions, it does seem significant that in 22 series of self- and cross-sterile pollinations not a single seed was set, whereas in the 6 fertile crosses the number of seeds per capsule averaged as follows:

Unopened bud	511.83
First flower	409.5
Second flower	521.67

These results indicate an absence of pseudo-fertility in *Nicotiana alata*, or at least in those strains of that species with which these investigations were carried out.

ABNORMAL POLLEN AND SELF-POLLINATIONS

During the course of the investigation, a microscopic examination of the pollen grains was made, and the fact was observed that among the hybrids some plants showed a greater per cent of abnormal pollen than others. The preliminary observations suggested that there was probably some correlation between the per cent of abnormal pollen, the degree of pseudo-fertility, and the amount of self-pollination in the history of the genetic strain concerned. Hence, the pollen grains of the various strains were studied, and in the case of each plant examined the diameters of 50 pollen grains, taken directly across the field of the microscope so as to insure a random sample, were measured by means of an eye-piece micrometer (see "Complete Data" for the resulting measurements).

Pollen of plants containing a considerable amount of small pollen grains likewise contains an approximately equal number of unusually large grains, since, due to the abortion of some of the grains, others are given the opportunity of an unusual degree of development. Hence, in considering the measurements of 50

grains, those plants with a low percentage of abnormal pollen have many grains near the mean diameter, with very few, if any, at the extreme. On the other hand, those plants with a high percentage of abnormal pollen have fewer grains of the mean diameter and more at the extremes than do those with a low percentage. Consequently, the relative per cent of abnormal pollen can be judged by the relative height and spread of the curves, which can in turn be measured by the standard deviation, σ. The standard deviation, then, can be used as a basis for comparison of the amount of abnormal pollen in any two groups of plants, and by this means it is possible to determine whether or not there is a significant difference between the two strains.

Class	Genetic families included
Selfed of selfed	LA, LC, LE
Selfed of crossed	CA, CB, CC, CD
Crossed of selfed	GOB
Crossed of selfed and crossed	GA, GC, GOF, GOX
Crossed of crossed	CL, CM, CG, CH, GE, GF, GOA, GOC, LH

Each group of families was considered as a unit. Frequency tables were made for each of them, and the standard deviation was calculated in every case with the following results.

Class	No. of grains	Standard deviation
Selfed of selfed	450	.9933 ± .0223
Selfed of crossed	475	1.0834 ± .0237
Crossed of selfed	350	.7032 ± .0179
Crossed of selfed and crossed	1500	.6098 ± .0075
Crossed of crossed	1300	.7296 ± .0097

The notation must be made here that the measurements in the selfed of crossed families and in a few of the crossed of crossed families were made early in the work and only 25 grains were measured in each plant. The latter have not been brought into this calculation, but since there were no measurements made on the selfed of crossed families during the latter part of the work, they are included here.

Since it was conceived that possibly the abnormal pollen was correlated with the degree of self-pollination in the history of the plants or with the recency of the self-pollination, the plants were divided into the following groups on this basis. The "selfed of

selfed" class came from seeds which were the result of self-pollinations made on plants which, in turn, were the results of self-pollinations. The "selfed of crossed" class came from self-pollinations on plants which were the product of a cross-pollination. The "crossed of selfed" class were plants which were the product of crosses between plants which, in turn, had come from self-pollinations. The "crossed of selfed and crossed" class were those individuals coming from crosses between two plants, one of which was the result of a self-pollination and the other the product of a cross between two parent plants. Finally, the "crossed of crossed" plants were the product of crosses between plants which, in turn, were the product of cross-pollinations.

To determine the significance of the difference it is simply necessary to divide the difference in the values of σ by the probable error of that difference, and if the quotient is greater than three the difference is significant.

$P.E._{diff.} = \sqrt{E_{\sigma_1}^2 + E_{\sigma_2}^2}$ where E_{σ_1} = probable error of σ in one group and E_{σ_2} is that of σ in the other. Therefore,

$$\frac{\text{Diff.}}{\text{P.E.}} = \frac{\text{Diff.}}{\sqrt{E_{\sigma_1}^2 + E_{\sigma_2}^2}}.$$

By means of this equation, the selfed of selfed group was compared with each of the other classes of plants with the following results:

Class	$\frac{\text{Diff.}}{\text{P.E.}}$
Selfed of crossed	2.77
Crossed of selfed	10.14
Crossed of selfed and crossed	16.32
Crossed of crossed	10.85

These results then indicate that there is no significant difference between the standard deviation in diameter of pollen grains in the selfed of selfed, and selfed of crossed classes. On the other hand, there is a very significant difference between that of the above 2 classes and that of the crossed of selfed, crossed of selfed and crossed, and crossed of crossed groups. As has been shown above, a significant difference in standard deviation indicates a

significant difference in the percentage of abnormal pollen. Hence, in the strains studied, it appears that the percentage of poor pollen in plants which are the immediate products of self-pollinations is significantly greater than that in those plants which are the immediate products of cross-pollinations. It is interesting that, although poor pollen is generally considered the result of hybridization, here, among hybrids, self-pollination greatly increases the percentage of abnormal pollen over that found in plants resulting from cross-pollinations.

Summary

1. In the *Nicotiana alata* × *N. Forgetiana* hybrids, pseudo-fertility is exhibited in both self- and cross-sterile combinations, and is of the same order in both cases.

2. It has been definitely demonstrated that in *Nicotiana* pseudo-fertility is not of the same order as true fertility, but that it does stand in direct relation to true sterility.

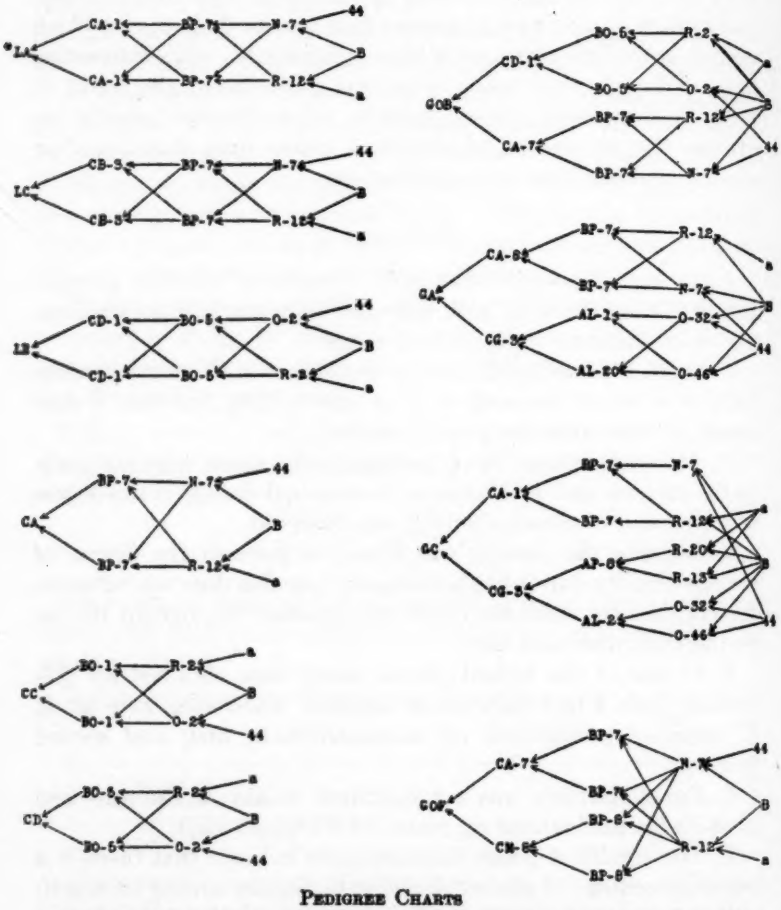
3. Although in some cases, perhaps, pollinations were not made at the extreme end of the season, no seasonal change in the degree of expression of pseudo-fertility was observed.

4. Bagging the panicle was found to increase the degree of pseudo-fertility, but it has been shown that this does not influence the conclusions, since the effect was constant throughout the investigations discussed here.

5. In one of the hybrid plants, seeds were obtained by pollinating buds 4 to 6 days before anthesis, when none were set in 17 series of pollinations on unopened buds, first, and second flowers.

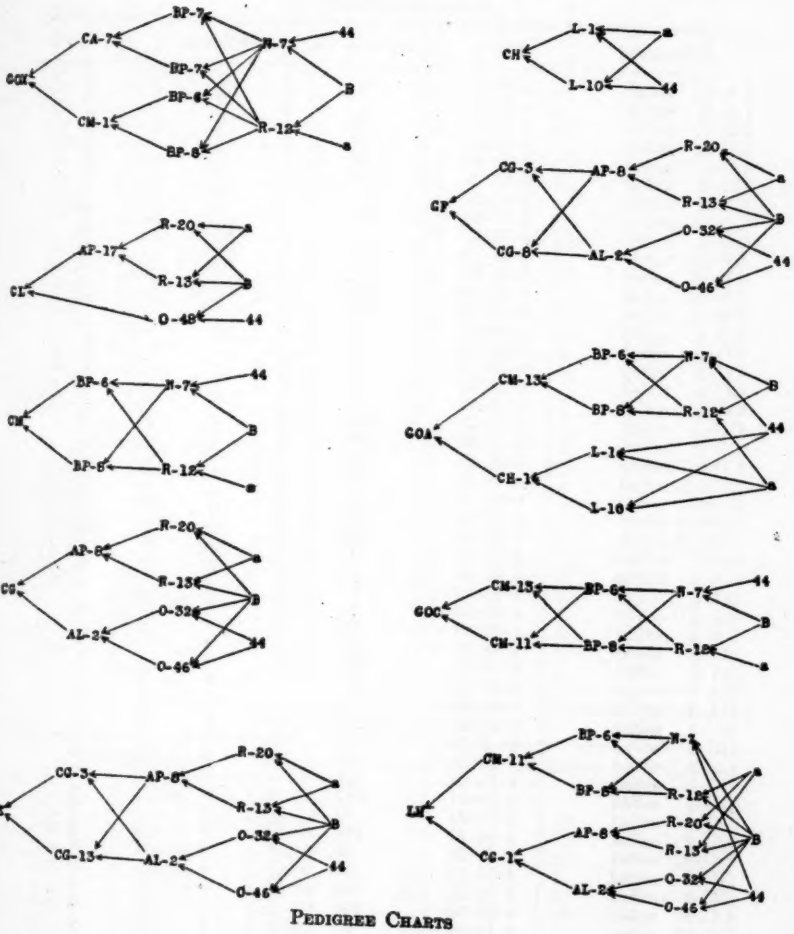
6. Pseudo-fertility was not exhibited in any of the self- and cross-sterile pollinations on plants of *Nicotiana alata*.

7. The results of pollen measurements indicate that there is a larger percentage of abnormal pollen in families arising from self-pollinations than in cross-bred strains.



PEDIGREE CHARTS

* The family LA is the result of a self-pollination on plant 1 of family CA, which, in turn, came from a self-pollination on plant 7 of family BP. The family BP, in turn, is the product of a cross between plant 7 of family N, and plant 12 of family R. These families have come from plants 44 and B, and B and a, respectively.



PEDIGREE CHARTS

TABLE VI

COMPLETE DATA

NICOTIANA ALATA × NICOTIANA FORGETIANA

Self-sterile pollinations

Pollination	Date	Unopened bud	1st flower	2nd flower
LA–1 selfed	4–28–24	0	0	0
LA–3 selfed	4–28–24	0	0	0
LC–1 selfed	4–14–24	37	0	0
LC–2 selfed	4–14–24	42	0	0
LC–9 selfed	4–28–24	40	0	0
CA–1 selfed	3–17–23	518	89	0
CA–3 selfed	3–30–23	?	30	0
CB–3 selfed	3–27–23	217	?	56
CB–7 selfed	3–31–23	?	282	26
CB–10 selfed	3–31–23	413	311	27
CB–13 selfed	3–31–23	?	28	81
CB–14 selfed	3–27–23	473+	136+	48+
CB–14 selfed	4–14–23	550	29	54
CC–1 selfed	4–26–23	0	0	0
CC–1 selfed	4–26–23	0	0	0
CC–5 selfed	4–26–23	0	0	0
CC–5 selfed	4–26–23	0	0	0
CC–9 selfed	4– 3–23	0	0	0
CD–1 selfed	4– 5–23	661	0	0
CD–4 selfed	4– 5–23	588	27	?
GOB–1 selfed	4–16–24	387	79	120
GOB–2 selfed	4–18–24	326	217	0
GOB–4 selfed	4–21–24	0	0	0
GOB–4 selfed	4–30–24	314	0	0
GOB–6 selfed	4–28–24	491	262	211
GOB–7 selfed	4–21–24	0	168	0
GA–1 selfed	4–21–24	0	0	0
GA–2 selfed	4–18–24	0	0	0
GA–2 selfed	4–28–24	0	0	0
GA–2 selfed	4–30–24	27	0	0
GA–3 selfed	4–28–24	0	0	0
GA–4 selfed	4–28–24	284	116	0
GA–5 selfed	4–30–24	0	0	0
GA–6 selfed	4–30–24	0	0	0
GC–1 selfed	3–29–24	48	0	0
GC–1 selfed	4– 8–24	14	0	0
GC–2 selfed	4–26–24	123	0	0
GC–3 selfed	4–14–24	124	0	0
GC–3 selfed	4–26–24	387	0	0
GC–4 selfed	4–14–24	62	0	0
GC–4 selfed	4–16–24	140	0	0
GC–5 selfed	4–14–24	29	0	0
GC–6 selfed	4–18–24	0	0	0
GC–6 selfed	4–26–24	149	0	0
GC–9 selfed	4–26–24	100	0	0
GC–12 selfed	4–18–24	0	0	0
GOF–2 selfed	4–21–24	75	0	0
GOF–3 selfed	4–18–24	0	0	0
GOF–3 selfed	4–30–24	0	0	0
GOF–4 selfed	4–28–24	0	103	0
GOF–4 selfed	4–30–24	4	4	13
GOF–5 selfed	4–21–24	0	0	0

Pollination	Date	Unopened bud	1st flower	2nd flower
GOX–1 selfed	4– 8–24	0	0	0
GOX–1 selfed	4–11–24	0	0	0
GOX–3 selfed	4–30–24	0	0	0
GOX–5 selfed	4–30–24	0	0	0
GE–1 selfed	4–21–24	0	0	0
GE–1 selfed	4–28–24	0	0	0
GF–1 selfed	4–16–24	0	0	0
GF–1 selfed	4–28–24	329	0	0
GF–1 selfed	4–30–24	0	0	0
GF–2 selfed	4–16–24	121	0	0
GF–2 selfed	4–28–24	0	0	0
GF–3 selfed	4–30–24	0	0	0
GF–5 selfed	4–30–24	0	0	0
GF–6 selfed	4–21–24	146	0	0
GF–7 selfed	4–21–24	31	371	0
GF–9 selfed	4–21–24	0	208	0
GF–9 selfed	4–28–24	0	0	0
GF–8 selfed	4–30–24	117	0	0
GOC–1 selfed	5– 1–24	0	0	0
GOC–3 selfed	4–30–24	76	0	0
CH–2 selfed	3–27–23	0	0	0
CM–12 selfed	4–14–23	0	0	0
GOA–1 selfed	4–11–24	0	0	0
GOA–1 selfed	4–14–24	0	0	0
GOA–1 selfed	4–30–24	0	0	0
GOA–2 selfed	4–16–24	419	154	0
GOA–2 selfed	4–30–24	7	0	0
GOA–3 selfed	4–18–24	0	0	0
GOA–3 selfed	4–30–24	0	0	0
GOA–4 selfed	4–21–24	0	0	0
GOA–4 selfed	4–30–24	0	0	0
LH–1 selfed	4–21–24	67	0	0
LH–1 selfed	4–28–24	45	79	0
CG–1 selfed	4– 6–23	0	0	0
CG–5 selfed	3–27–23	24	0	0
CG–9 selfed	4– 7–23	54	0	0
CG–10 selfed	3–27–23	0	0	0
CG–18 selfed	4– 5–23	?	?	0
CG–20 selfed	4– 7–23	?	0	0

Cross-sterile pollinations

Pollination	Date	Unopened bud	1st flower	2nd flower
LC–2 × LC–4	4–29–24	72	0	0
CA–3 × CA–1	3–30–23	?	46	0
CA–3 × CA–4	3–30–23	?	69	0
CA–13 × CA–11	3–25–23	378	307	155
CA–4 × CB–13	4–19–23	338	0	55
CA–4 × CB–13	4–19–23	158	0	0
CA–6 × CB–4	4–10–23	?	195	314
CA–6 × CB–9	4–10–23	385	0	0
CA–11 × CB–15	3–27–23	379	231	73
CA–12 × CB–9	4–10–23	250	0	0
CB–7 × CB–5	3–31–23	?	0	0
CB–7 × CB–14	3–31–23	?	98	?
CB–12 × CB–6	3–27–23	?	?	0
CB–13 × CB–10	4– 5–23	0	0	0

Pollination	Date	Unopened bud	1st flower	2nd flower
CB–13 × CB–14	3–31–23	21	0	0
CB–14 × CB–15	4– 5–23	538	276	0
CB–14 × CA–4	4–14–23	418	31	?
CC–1 × CC–5	4–26–23	355	390	338
CC–1 × CC–5	4–26–23	461	381	261
CC–5 × CC–1	4–26–23	0	0	0
CC–5 × CC–1	4–26–23	0	?	?
CC–6 × CC–9	4–26–23	0	164	0
CC–7 × CC–9	4– 3–23	?	0	0
CC–8 × CC–7	4– 7–23	261	?	0
CC–9 × CC–7	4– 7–23	0	0	205
CD–1 × CD–2	4– 5–23	536	0	?
GA–3 × GA–4	4–18–24	0	0	0
GA–3 × GA–6	4–28–24	0	0	0
GA–5 × GA–2	4–28–24	194	0	0
GA–6 × GA–4	4–28–24	257	0	0
GA–15 × GA–16	4–30–24	0	0	0
GC–3 × GC–4	4–18–24	183	?	0
GC–5 × GC–7	4–21–24	248	0	0
GC–9 × GC–4	4–26–24	0	0	0
GF–7 × GF–9	4–28–24	530	136	47
GF–9 × GF–18	4–30–24	8	0	0
GOC–1 × GOC–3	4–30–24	0	0	0
GOC–3 × GOC–1	4–30–24	63	0	0
CM–8 × CM–3	4– 7–23	461	0	0
CM–8 × CM–6	4– 7–23	141	?	0
CM–11 × CM–3	4–14–23	250	0	0
CM–12 × CM–5	4–14–23	0	0	0
CG–2 × CG–10	4– 3–23	424	0	0
CG–5 × CG–10	3–27–23	?	?	?
CG–6 × CG–5	4– 7–23	?	0	0
CG–6 × CG–8	4– 7–23	0	0	23
CG–8 × CG–10	4– 5–23	470	41	32
CG–9 × CG–1	4– 3–23	0	0	0
CG–10 × CG–2	3–27–23	0	0	0
CG–10 × CG–2	4– 3–23	0	0	0
CG–18 × CG–8	4– 7–23	?	0	0
CG–19 × CG–4	4 –5–23	0	0	0
CL–3 × CL–5	4– 7–23	36	0	0
CL–4 × CL–10	4– 5–23	72	0	0
GOA–1 × GOA–2	4–23–24	0	0	0
GOA–2 × GOA–4	4–26–24	322	0	10
GOA–3 × GOA–1	4–26–24	0	0	0
GA–5 × GC–6	5– 1–24	0	0	0
GE–1 × GOF–1	4–28–24	4	0	0
GF–7 × GC–9	4–30–24	0	0	0
GOA–1 × GE–1	4–23–24	11	0	0
GOA–4 × GOB–1	4–26–24	289	0	?
GOA–1 × GOX–1	4–18–24	120	0	0
GOB–1 × GOA–4	4–26–24	161	0	0
GOC–1 × GOA–2	4–26–24	0	0	0
GOC–1 × GOX–1	4–26–24	0	0	0
GOF–5 × GOX–3	4–28–24	0	0	0
GOX–1 × GOA–4	4–26–24	0	0	0
LA–1 × LC–6	4–28–24	243	0	0
LH–1 × GI	4–28–24	76	37	409
LH–1 × LA–1	4–28–24	0	0	0
LH–1 × LC–5	4–28–24	75	0	0
CB–4 × CG–8	4–19–23	230	87	41

Pollination	Date	Unopened bud	1st flower	2nd flower
CB-4 × CG-8	4-19-23	292	105	0
CC-1 × CL-4	4-26-23	?	413	26
CC-5 × CL-4	4-26-23	0	0	0
CC-5 × CL-4	4-26-23	124	?	0
CD-4 × CG-20	4- 5-23	519	0	0
CG-20 × CL-4	4-19-23	334	0	0
CG-20 × CL-4	4-19-23	0	0	0
CH-1 × CM-6	3-31-23	?	0	0
CM-12 × CH-2	4-14-23	0	0	0
CG-5 × CB-10	3-27-23	0	0	0

Cross-fertile pollinations

Pollination	Date	Unopened bud	1st flower	2nd flower
LC-4 × LC-3	4-28-24	48	?	216
LC-5 × LC-1	4-28-24	422	153	187
GOB-7 × GOB-3	4-28-24	540	?	390
GA-3 × GA-15	4-28-24	371	274	?
GA-6 × GA-8	4-28-24	342	378	285
GA-6 × GA-15	4-28-24	357	343	225
GC-5 × GC-9	4-16-24	385	437	430
GC-9 × GC-3	4-26-24	522	222	627
GOF-3 × GOF-1	4-21-24	393	320	279
GOF-5 × GOF-13	4-30-24	181	245	430
GOX-1 × GOX-4	4-26-24	359	411	502
GOX-3 × GOX-4	4-26-24	288	475	511
GF-1 × GF-10	4-28-24	291	396	379
GF-7 × GF-10	4-30-24	528	646	591
GF-9 × GF-10	4-28-24	494	390	369
CM-1 × CM-3	4- 7-23	689	669	0
CM-1 × CM-12	4- 7-23	0	666	0
CM-1 × CM-14	4- 7-23	254	455	321
CM-11 × CM-14	4- 7-23	500	?	409
CG-3 × CG-8	4- 7-23	301	248	243
CG-5 × CG-7	4- 3-23	362	334	410
CG-6 × CG-1	4- 3-23	838	969	825
CG-18 × CG-9	4- 5-23	?	530	488
CL-2 × CL-1	4- 7-23	481	515	455
CL-5 × CL-10	4- 7-23	467	458	418
CL-8 × CL-1	4- 7-23	311	780	713
CL-8 × CL-6	4- 7-23	787	834	724
CL-10 × CL-2	4- 7-23	321	396	446
GC-3 × GOA-1	4-14-24	433	685	703
GOB-1 × GA-1	4-30-24	293	422	295
GOE-1 × GOF-1	4-18-24	381	171	101
GOF-4 × GOA-4	4-28-24	234	80	341
CA-3 × CH-1	3-30-23	?	673	327
CB-14 × CH-2	4-14-23	444	396	484
CB-14 × CM-12	4-14-23	246	256	247
CB-15 × CM-12	3-27-23	315	211	0
CD-1 × CA-1	4- 5-23	1004	873	908
CD-4 × CL-5	4- 5-23	681	398	599
CD-4 × CM-6	4-14-23	257	570	496
CF-3 × CH-1	3-27-23	619	343	515
CG-20 × CA-1	4- 7-23	511	577	446
CH-1 × CG-18	3-31-23	757	586	649
CH-2 × CA-4	4-14-23	388	395	?
CH-2 × CB-14	4-14-23	356	377	342
CH-2 × CL-6	4-14-23	440	439	429
CM-11 × CG-1	4- 3-23	?	553	?
CM-12 × CA-4	4-14-23	424	646	24
CM-12 × CB-14	4-14-23	750	612	431
CM-12 × CL-6	4-14-23	500	717	627

NICOTIANA ALATA			
Self-sterile pollinations			
Pollination	**Date**	**Unopened bud**	**1st flower**	**2nd flower**
CJ–3 selfed	4– 3–23	0	0	0
HA–3 selfed	4–18–24	0	0	0
HA–3 selfed	5– 1–24	0	0	0
HA–4 selfed	4–29–24	0	0	0
HA–5 selfed	4–21–24	0	0	0
HB–1 selfed	4–19–24	0	0	0
HC–2 selfed	4–21–24	0	0	0
HC–2 selfed	4–29–24	0	0	0
HC–3 selfed	4–29–24	0	0	0
HI–1 selfed	4– 8–24	0	0	0
HI–2 selfed	4–14–24	0	0	0
HI–3 selfed	4–14–24	0	0	0
HI–3 selfed	5– 1–24	0	0	0
HI–4 selfed	4–16–24	0	0	0
HI–5 selfed	4–18–24	0	0	0
HI–8 selfed	4–21–24	0	0	0
Cross-sterile pollinations				
CJ–1 × CJ–3	4– 3–23	0	0	0
CJ–2 × CJ–1	4– 3–23	0	0	0
HA–5 × HA–6	4–29–23	0	0	0
HC–2 × HC–1	4–14–24	0	0	0
HC–2 × HC–1	4–29–24	0	0	0
HI–2 × HI–9	4–29–24	0	0	0
Cross-fertile pollinations				
HA–1 × HA–5	4–29–24	679	600	605
HA–5 × HA–3	4–29–24	600	663	500
HA–6 × HA–3	4–29–24	637	737	831
HC–1 × HC–5	4–29–24	155	446	258
HC–2 × HC–4	4–29–24	1000	13	936
HA–1 × HI–9	4–18–24	787	654	773
HA–5 × HI–2	4–24–24	900	1569	1079
HB–1 × HC–1	4–21–24	450	500	1000

TABLE VII

POLLEN MEASUREMENTS (IN TERMS OF DIVISIONS ON MICROMETER SCALE; 1 DIVISION = 8 MICRA)

	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
CA–7					4		17		3		1		
CA–13					4		16		4		1		
CB–1			2		4		13		5		1		
CB–4			3		3		14		3		2		
CB–5					7		8		10				
CB–7	1		6		6		5		6		1		
CC–1					3		11		7		4		
CC–2	1		4		3		10		4		2		1
CC–3			1		1		10		8		3		2
CC–4					2		14		9				
CC–5			1		1		16		5		2		

	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
CC–8			2		3		7		7		6		
CC–10			1		3		8		10		3		
CC–11			1		3		17		3		1		
CD–1	1		5		2		8		9				
CD–2	2		4		3		13		3				
CD–3	1		4		7		13						
CD–4	3		6		4		10		2				
CD–8	1		1		4		15		4				
CL–4					3		8		13		1		
CL–7					9		16						
CL–8			3		6		16						
CL–11					3		22						
CM–1					2		20		3				
CM–6					2		21		2				
CM–10					1		19		5				
CM–13					2		19		4				
CG–20					9		16						
CH–1					1		22		1			1	
GA–1			1	6	14	20	8	1					
GA–2			1	3	15	25	6						
GA–3				4	8	22	15	1					
GA–4			1	1	13	23	10	2					
GA–5			1	4	2	27	14	2					
GA–6			2	3	7	22	14	2					
GA–8				1	4	20	24	1					
GA–15				2	7	23	16	2					
GA–16			2	3	10	19	13	3					
GC–2					10	16	24						
GC–3				1	10	17	19	3					
GC–4			3	7	15	11	14						
GC–6				3	6	16	25						
GC–7	2		5	7	14	17	5						
GC–9	3		5	1	10	10	19	2					
GC–13			2	1	10	10	26	1					
GE–1	3		2	2	6	17	20						
GF–1				1	10	23	16						
GF–2	2		3	2	3	23	16	1					
GF–3				2	5	13	26	4					
GF–4	2		3	3	4	22	7	4	5				
GF–5			3	6	8	25	7	1					
GF–6	1		1	2	4	15	25	2					
GF–7			1	2	3	20	22	2					
GF–8				1	6	18	25						
GF–9					6	28	16						
GF–10				2	4	23	20	1					
GF–11			1	2	4	26	17						
GF–18		1	1	1	3	24	20						
GOA–1		1	1	2	8	25	12	1					
GOA–2			6	8	11	12	11	2					

	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
GOA-3				2	10	17	20	1					
GOA-4		1	3	10	13	15	7	1					
GOA-5			2	2	2	10	21	10	1	1	1		
GOA-6	8	20	8		1	5	7		1				
GOA-7				1	3	8	18	19	1				
GOB-1				1	2	26	20	1					
GOB-2			1	1	10	25	12	1					
GOB-3		2	7	3	6	10	20	2					
GOB-4		4	7	1	4	11	22	1					
GOB-6	1	2	3	4	6	19	12	2	1				
GOB-7			3	4	4	17	19	3					
GOB-8		2	3	4	6	17	15	2	1				
GOC-1		3	2	2	6	17	20						
GOC-3			1	1	6	21	17	3	1				
GOC-10				5	11	24	9	1					
GOC-11			1	2	4	7	18	17	1				
GOF-1					13	27	9	1					
GOF-3	4	2	5	3	3	22	11						
GOF-4			1	4	17	24	2	1	1				
GOF-5	2	5	1	1	13	23	5						
GOF-6			2	4	17	11	9	6	1				
GOF-7	4	3	1	2	22	16	2						
GOF-11			1	2	4	26	17						
GOF-13	4	1	4	3	19	19							
GOX-1				3	10	18	19						
GOX-2			2	2	10	21	14	1					
GOX-3			1	2	2	20	15	7	3				
GOX-4			1	2	5	22	17	3					
GOX-5				6	10	22	12						
GOX-6				1	10	20	19						
LA-1	2	1	2	1	4	13	14	6	5	2			
LA-3	2	1	2	2	1	15	19	5	2	1			
LA-4	3	4	5	5	5	7	17	3	1				
LC-5	2	2	2	5	4	10	13	3	4	3	1	1	
LC-6	2	3	3	5	7	13	11	2	2	1	1		
LC-8		3	5	6	7	9	10	4	4	1	1		
LC-9		3	3	4	4	11	15	4	3	1	1	1	
LE-3	1	1	2	1	5	18	17	3	2				
LE-5				3	11	25	11						
LH-1	3	5	7	5	6	13	10	1					
LH-3	1	3	5	5	8	18	10						

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